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American Dairy Science Association®

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ADSA-SAD Dairy Foods Oral Competition

1 The surprising benefits of camel milk. G. M. DeMers* and D. R. Olver, *Pennsylvania State University*, *University Park, PA*.

With an ever-growing list of alternatives to traditional cow milk being offered in grocery stores everywhere, there is a new contender in this evolving market: camel milk. In fact, a leading food and beverage website declared camel milk to be one of the top 5 trends shaping the international dairy industry in 2019. With a flavor profile more similar to cow milk than other many other choices (such as plant-based alternatives), camel milk contains many of the essential nutrients associated with cow milk while retaining the true "milk" title. Two species of camels, Dromedary and Bactrian, produce milk that offers additional nutritional benefits attractive to some segments of consumers. Studies of people consuming camel milk have demonstrated lower instances of lactose intolerance reactions, lower blood sugar levels with improved insulin sensitivity, and increase immune support. According to the Food and Agricultural Organization of the United Nations, typical camel milk contains 3.1% protein and 3.5% fat. However, there is considerable variation in composition data for some constituents. High levels of camel milk components such as lactoferrin contribute to its antibacterial and antifungal properties. In addition to these benefits, camels are very efficient animals that can produce more milk from poorer quality feedstuffs compared with other species. One disadvantage of camel milk is that traditional dairy products such as butter and cheese are more difficult to manufacture, leading to a mainly fluid-based market. Camel milk is primarily produced in Africa and Asia, with Somalia being the largest producer of camel milk worldwide. In the United States, several Amish and Mennonite farms have begun marketing camel-based dairy products. As the availability of camel milk continues to grow, it has the opportunity to become a growing contender in the dairy aisle.

Key Words: camel milk, diabetes, immunity

2 Evaluating the impact of novel products to the dairy market on fluid milk utilization. A. Crews* and J. Bohlen, *University of Georgia*, *Athens, GA*.

In the last decade, the consumption of fluid milk has continued to drop in the United States as consumer preference for milk substitutes continues to rise. With increasing health concerns and diet trends, remaining consumers of fluid milk are pressuring for innovation in the market of dairy food production. Gallon jugs of milk are less desirable with smaller families and a short shelf stability limit its demand, especially when habitual breakfast is becoming less popular and the market for its complimentary good, cereal, is declining. It begs the question as to why fluid milk remains so highly regarded by cooperatives in regions such as the Southeast. Simply put, the mechanics and labor processing facilities value at too large of an expense to give reason to push for investment in an area such as the Southeastern United States with such an unsteady seasonal milk supply. Across the US but especially for producers in the southeastern United States, the need for increased fluid milk utilization is called for. This need for price increase and stabilization for dairy producers coupled with consumer driven markets ultimately calls for the reinvention of standard fluid milk. Novel products changing fluid milk utilization have focused on its inclusion in other products, refinement of the milk product, and more attractive packaging. These products include items such as milk-based coffee products to target a widely consumed product, A2 milk, and milk presented in glass bottles. Ultimately, the fluid milk industry will need to diversify its product offering and methodologies of presentation to remain profitable, and refinement and packaging offer opportunities for stabilization of dairy prices.

Key Words: fluid milk, market innovation, utilization

3 Effects of pasteurization method on milk composition. L. M. Adams*, G. Mazon, and J. H. C. Costa, *University of Kentucky*, *Lexington, KY*.

From 1998 to 2003, 75% of disease outbreaks related to milk consumption occurred in states where raw milk sales are permitted (Lucey, 2015). However, illnesses associated with milk consumption totaling less than 1% of foodborne illnesses because of the pasteurization process (FDA, 2011). The main goal of pasteurization is to decrease pathogens such as *Salmonella*, *Campylobacter*, and *E. coli* in raw milk to a level safe for consumption (Oliver et al., 2005). There are 3 main pasteurization methods: low-temperature long time (63°C for 30 min), high-temperature short time (HTST; 72°C for 15 s), and ultra-high temperature (UHT; 135 to 150°C for 1 to 2 s). According to the International Dairy Foods Association, HTST pasteurization is the most common method used in the United States but yields milk with a lower shelf life than UHT (Bezie et al., 2019). Although UHT pasteurization extends milk shelf life, it also affects milk protein and lipid composition. In the UHT process, the extreme heat exposure denatures proteins at the tertiary and secondary structure level of whey proteins (Qi et al., 2015). Complete proteolysis occurs in the native milk plasmin and bacterial proteinases of UHT processed milk (Datta and Deeth, 2003). Moreover, the process of proteolysis can result in gelatinization of milk leaving a residue and a slight change in flavor when comparing UHT to HTST milk (Meunier-Goddik, 2016). Regarding lipids, research indicates that triglyceride levels are significantly lower in UHT compared with HTST and free fatty acid concentrations increased indicating lipolysis in UHT milk (Xu et al., 2019). Research suggests that those slight changes to milk composition might affect the acceptance of UHT milk by consumers as evidenced by a sensory panel in which HTST milk was liked slightly more than UHT (Chapman and Boor, 2001). In summary, consumer's preferences should be considered when selecting a pasteurization method for milk as it can affect the protein and lipid structure in milk consequently affecting and taste perception.

Key Words: food safety, pathogen, sensory analysis

ADSA-SAD Dairy Production Oral Competition

4 Gene sequencing of embryos. C. McGehee*, R. Cockrum, and D. Winston, *Virginia Tech*, *Blacksburg, VA*.

Before 2009 it required nearly 5 years before the genetic merit of an individual animal was determined. Once genomics was introduced into national evaluations, genetic progress in the dairy industry rapidly increased. The genetic merit of an animal can be determined at a young age and is highly reliable due to the large reference population available. However, the majority of genetic gain has been achieved on the sire side, resulting in genetic lag of cows. One approach to increasing the genetic gain of females is through embryo genotyping. This technology allows producers to make more educated decisions on which embryos to implant. Genotyping embryos, therefore, increases genetic progress by increasing selection intensity. This technology can also identify embryonic defects and genetic recessives to reduce early embryonic loss. Two techniques can be used to acquire biopsies from embryos for genomic testing. The first method is blade biopsy, which can be used for embryos in the late-morula or blastocyst stage sampling cells from the trophectoderm. The second method is ideal for cleavage-stage or morula-stage embryos. This method utilizes a needle to aspirate cells from the embryo; it requires that enough cells be collected to complete gene testing but must ensure there are not too many taken that will compromise the continued development of the embryo. Because sufficient DNA cannot be obtained to conduct a SNP-chip genotyping, enzymatic pre-amplification of the biopsy is required. However, fertility of the genotyped embryos is still a concern. Single-cell gene sequencing is a technology that will be available in the future, which would increase the integrity of the embryos and further the genetic improvement of dairy cows.

Key Words: embryo, genomic selection, embryo biopsy

5 Manipulating circadian rhythms through controlled light-dark phases in the prepartum period on cow performance in the next lactation. A. Rauton* and J. Bohlen, *University of Georgia*, *Athens, GA*.

Circadian rhythms, derived from the Greek “circa” meaning about and “dian” meaning day, describe a cycle over a 24-h period, which dictate sleep and wake cycles in most biological organisms. Although many aspects of these rhythms are genetically determined, it has been proven that they can be manipulated by means of food availability, temperature and light. Studies involving circadian rhythms in the dairy cow began with correlations between day length and milk production but have since expanded to include the impact these rhythms have on metabolic parameters, daily activity and hormone production. Since that first development of the positive impact of 16 h of light and 8 h of dark on milk production, most studies have focused their work on impacts during lactation. However, little is known regarding the role of circadian rhythm cycles from the end of a lactation to the beginning of the next. This transition period in dairy cattle means major hormonal shifts and changes in metabolism in an effort to accommodate the energetically demanding onset of lactation. Recent studies have shown that using a phase shifted model of the standard light dark period has the ability to positively impact these changes. This stimulation pattern involves shifting the light and dark phases by 6 h periodically throughout the treatment period. This shift, combined with circadian rhythm timing and synchrony, has been shown to alter many animal processes to include attenuation of melatonin production as well as body temperature. More consistent melatonin levels throughout the transition period prompt the animal to spend more time resting as opposed to being more active while attenuation of body temperature decreases the amount of energy used for heating and cooling throughout the day. Ultimately these culminate in a decrease in energy spent in the time before calving which results in an improvement in milk yield and fat content during the next lactation. These results indicate that by interrupting a cow’s naturally occurring rhythm during the transition period, a producer may be able to alter that cow’s performance in her next lactation. These novel insights into the manipulation of circadian rhythms during the transition period offer a new and innovative way to care for lactating cows before the start of lactation.

Key Words: circadian rhythm, transition, lactation performance

6 Pain management for disbudding dairy calves. A. K. Bitter* and C. C. Williams, *Louisiana State University*, *Baton Rouge, LA*.

Disbudding and dehorning calves are common practices in the dairy industry. Disbudding is the removal of the horn before it attaches to the frontal sinus. The most common practice of disbudding is with an electric hot iron. Another common method of disbudding is chemical disbudding via caustic paste, and a less common practice of disbudding is by amputation. Once the horn begins to attach to the skull around 2 mo of age, then disbudding is no longer an option. Dehorning is the process of removing the horn when it fuses to the frontal sinus. There are different methods of dehorning including guillotine dehorner, surgical wire, horn saw, Barnes dehorner, or tippers. Dehorning is a more painful procedure because of the fusion of the horn to the tender sinus above the eyes. In both procedures, the calf experiences pain and discomfort. While these procedures are painful to calves, some reasons for disbudding or dehorning include decreasing the risk of injury to other animals and reducing the risk of injuring workers. Disbudding or dehorning a calf is a painful process, and because of this, more dairy farms are beginning to use short and/or long-term pain management for this procedure to practice better management in their dairy operation. The best time to disbud a calf is from one to 6 weeks of age because the horn is not yet attached to the skull; therefore, it is less painful for the calf. There are also various ways to control pain caused by disbudding or dehorning procedures. Research has shown that the use of non-steroidal anti-inflammatory drugs (NSAIDs), local anesthetics, and sedation alleviates the pain calves may experience during and after disbudding. Research has also shown that pain management during disbudding results in improved growth and performance of calves. In order for dairy operations to practice better management practices and increase their level of welfare of their animals, they should disbud their dairy calves from one to 6 weeks of age and use NSAIDs and local anesthesia when disbudding or dehorning.

Key Words: dairy calves, disbudding, pain management

7 Nutritional management of subacute ruminal acidosis in early lactation. M. Mosher* and E. Eckelkamp, *University of Tennessee Institute of Agriculture*, *Knoxville, TN*.

Proper nutrition is key to early lactation management, particularly for subacute ruminal acidosis (SARA). Early lactation is one of the most energy demanding periods of a cow’s life. Early lactation marks the end of gestation and parturition and the onset of lactation. Subacute ruminal acidosis is typically defined as the rumen pH dropping below the ideal pH range (6.0 to 6.4) to $\leq$5.6 for $\geq$3 h. Low pH can lead to insufficient rumen buffering and decreased production and health issues including: laminitis, gastrointestinal damage, and liver abscesses. Cows with SARA do not exhibit clinical signs leading to missed cases, late diagnosis, or only diagnosing severe cases. Cows with SARA cost the US dairy industry $500 million to $1 billion annually through decreased milk production, decreased milk fat percentage and overall milk quality, and cow mortality. Although treatment is key to all disease management, prevention is key with SARA. Effective SARA prevention relies on proper ration formation. Producers who maintain a forage to grain ratio of 60:40, a high starch percentage, and a low amount of forage neutral detergent fiber can maintain high production with a lower risk of SARA. A grain ratio $\geq$50% can increase SARA risk and depress milk fat percentage. Too much grain in the diet can lead to excess or abnormal production of volatile fatty acids which can decrease rumen pH. Particle size should also be considered. Particles that are too long

(>19 mm) or too short (<4 mm) can lead to an increased risk of SARA. Suggested particle size and distribution were ≤8% of ration ≥19mm, 50% of ration between 7mm to 9mm, 20% of ration between 4mm to 7mm, and 30% of ration ≤4mm. Providing a rumen buffer can also help control SARA, such as sodium sesquicarbonate and sodium bicarbonate. Rumen buffers help stabilize the rumen environment and can be used to bring rumen pH back to ideal levels. Through proper nutrition, control of subacute ruminal acidosis can be increased along with cow health and productivity.

Key Words: early lactation, subacute ruminal acidosis, nutrition

8 Precision and accuracy of mid-infrared spectroscopy for milk urea nitrogen analysis. E. M. Wood*, M. Portnoy, D. M. Barbano, and K. F. Reed, *Cornell University*, *Ithaca, NY.*

High levels of urea in blood, milk, and urine have been linked to poor nitrogen efficiency, increased feed costs, poor reproductive performance and increased environmental impact of dairy farming. Bulk tank average milk urea nitrogen (MUN) is often used to manage herd nitrogen efficiency. Current recommendations suggest MUN should be between 8 and 14 mg/dL to maintain milk production and reduce nitrogen losses, but a previous study found that commercial analysis of MUN ranged from 6.5 to 14.9 mg/dL for the same sample set. The objective of this study was to evaluate the precision and accuracy of milk testing lab MUN measurements. Milk samples were collected from multiparous Holstein cows (n = 16) 3 times daily (06:00, 14:00, and 22:00) over 7 consecutive days during early lactation (average DIM of 40 d). Samples were sent for analysis at 2 labs (A and B). Both labs tested samples using mid-infrared spectroscopy (MIR). Lab B also employed an enzymatic spectrophotometric method for measuring MUN on de-fatted and de-proteinated milk, here considered to be the gold standard protocol. The mean (sd) of MIR MUN for Lab A and B are 8.05 (1.33) and 6.41 (1.78) mg/dL respectively. The differences between the MIR MUN values and the enzymatic assay MUN for each lab were calculated and regressed on mean-centered enzymatic MUN values in a mixed model with random effect for day. Results of the linear regression are presented below. Labs A and B had significant ($P < 0.01$) negative slope coefficients, indicating MIR methods over predict MUN at low values and underpredict at higher values. The intercept estimates suggest Lab A significantly ($P < 0.01$) over predicts MUN by 2 mg/dL while Lab B tends ($P < 0.10$) to overpredict MUN by less than 0.5 mg/dL. Estimates of the residual variance and random effect of date indicate similar precision between labs. In spite of existing bias, results show the MIR accuracy has improved, however, sampled data is below industry average MUN and further work on samples with a wider range of MUN is required.

Table 1 (Abstr. 8). Parameter estimates of mixed model regression of MUN differences

Laboratory	β0	β1	σDay	σResidual
A	2.02 (0.173)	−0.555 (0.0639)	0.317	1.44
B	0.481 (0.218)	−0.512 (0.0694)	0.422	1.56

Key Words: MUN, spectroscopy

9 Evaluating the use of pulse oximetry, lactate levels, and lung ultrasounds in predicting respiratory illness in dairy calves. M. Hillis*, M. Gray, and J. Bohlen, *Animal and Dairy Science, University of Georgia*, *Athens, GA.*

The future productivity of a dairy operation is found in the calf barn. Growth and health of dairy calves represent a large expense to dairy producers but one, if done correctly, will provide dividends when lactation begins. The most commonly cited health issues in dairy calves are scours and respiratory diseases with the latter at times being the more elusive to identify. The objective of this study was to investigate new methodologies to ascertain calves at risk for respiratory diseases as well as accurately diagnosing respiratory illnesses. For this study we evaluated Holstein calves (n = 12) for blood lactate levels and pulse oximetry within 6 h of calving and then at 24-h intervals until 3 d of age to assess animals potentially predisposed to respiratory issues. Research shows correlation between high lactate concentrations and neonatal asphyxia in cases of dystocia, while pulse oximetry has long been used as an indicator of respiratory distress in humans and small mammals. Lung ultrasounds, using the 6-point ultrasound lung lesion scoring system, were performed on Holstein heifer calves (n = 15) weekly to identify emerging respiratory issues. Auxiliary health data such as dystocia score, weight, height, and signs of respiratory illness according to the Wisconsin Respiratory Scoring System was also collected. Lactate levels ranged from 4 – 5.2 mmol/L while pulse oximetry ranged from 84 to 98% spO2. Lactate and oxygen saturation were not associated with dystocia score ($P > 0.05$) nor correlated to incidence of respiratory distress or growth rate of calves ($P > 0.05$). Only one issue of respiratory distress was identified according to lung ultrasounds and respiratory scoring system thus no conclusive data may be elucidated from this part of the study. Limited respiratory illnesses in the current study impeded the ability to collect worthwhile data related to early and accurate identification of respiratory disorders. However, information regarding methodologies for lung ultrasound and how to use pulse oximetry in calves may prove useful in communications with producers.

Key Words: lung ultrasound, calves, respiratory

415 Impacts of exogenous estradiol on mammary collagen distribution in Holstein heifers. C. T. Oduyelu*, C. L. M. Parsons, A. J. Geiger, R. M. Akers, and K. M. Daniels, *Virginia Tech*, *Blacksburg, VA.*

Mammary collagen distribution is dynamic during growth. The objective was to determine effects of exogenous estradiol on mammary collagen distribution in Holstein heifers fed an enhanced (EH) diet. Twelve Holstein heifers (6 ± 2d old) were fed milk replacer (28.9% CP, 26.2% fat, DM basis), at 1.08 kg powder DM/d. Starter (26% CP; DM basis) was offered beginning at 4wk of age. Heifers received ear implants containing either estradiol (EH-E_2; n = 6; 25.7 mg estradiol) or placebo (EH; n = 6) at weaning (8wk). Heifers were slaughtered at 10wk. Mammary samples were collected from 2 zones within front right mammary glands (EH-E_2, n = 6; EH, n = 4), formalin fixed, and paraffin embedded. Zone 1 (Z1) was the parenchymal area immediately above the gland cistern. Zone 3 (Z3) was the interface of mammary parenchyma and mammary fat pad. Five-µm thick sections were stained with Picro-Sirius Red and Picro-Fast Green. Four to 6 total matched images per heifer and zone were obtained under both brightfield and polarized monochrome conditions using 20x objective lens magnification. A mask of epithelial areas was created per brightfield image with Image ProPlus (version 7; Media Cybernetics). Cell Profiler (version 2.1.1; www.cellprofiler.org) used the mask and monochrome images to measure stromal collagen birefringence. Dependent variables for each image were: total stromal area, stromal collagen area, and collagen as a percent of stromal area. Data were analyzed with a statistical model that included fixed effects of treatment, zone, and their interaction; heifer within treatment was the random effect. Total stromal area did not depend on treatment, zone, or the interaction. Stromal collagen area was not impacted by interaction or treatment but did depend on zone ($P < 0.01$). Stromal collagen area was 25,902 ± 1461μm^2 in Z1 and 16,159 ± 1461μm^2 in Z3. Percent of stromal area occupied by collagen was likewise affected by zone ($P < 0.01$), averaging 11.8 ± 0.7% in Z1 and 7.2 ± 0.7% in Z3. Overall, collagen deposition in mammary stroma was not impacted by exposure to exogenous estradiol but was dependent on sampling zone, with established areas (Z1) having more collagen than active growth regions (Z3).

416 Effect of a live yeast supplement and altered ruminal fermentability of dietary starch on the yields of milk and milk components of mid- to late- lactation dairy cows. U. Abou-Rjeileh*, A. N. Negreiro, and A. L. Lock, *Michigan State University*, *East Lansing, MI.*

We evaluated the effect of a live yeast supplement and changes in ruminal fermentability of dietary starch on feed intake and the yields of milk and milk components of mid- to late-lactation dairy cows (158 ± 76 DIM). Thirty-two multiparous Holstein cows (45.5 ± 5.1 kg/d) were used in a crossover design with two 28-d periods. A fermentable starch challenge (FSC) on the last 7 d of each period was utilized as a split-plot within period. Cows were blocked by milk yield and randomly assigned to 1 of 2 treatments. Treatments were: 1) control diet with no live yeast supplementation (CON); and 2) diet supplemented with a live yeast supplement (*Saccharomyces cerevisiae* from Phileo by Lesaffre) at 0.03% diet DM (YEAST). Diets were formulated to contain (% DM) 19.8% NDF, 28.8% starch, 16.9% CP, and 4.5% fat. Diets containing a 2:1 ratio (DM basis) of dry ground corn (DGC) to high-moisture corn (HMC) were fed from d 1–21 of each period. During the last 7 d of each period the ratio of DGC to HMC was switched to a 1:2 ratio to increase ruminal starch fermentability. Response variables were averaged for d 17–21 (before the FSC period) and d 27 and 28 (during the FSC period). The statistical model included the random effect of cow and fixed effects of treatment, period, challenge, and their interactions. There was no effect of treatments on DMI or the yields of milk and milk components (all $P > 0.13$). While there was no main effect of the FSC on DMI or the yields of milk and milk components (all $P > 0.30$), effects on milk fat content and yield varied for CON and YEAST. During the FSC, milk fat yield decreased from 1.69 to 1.62 kg/d for CON but was not different for YEAST (1.68 to 1.66 kg/d; interaction $P = 0.06$). Milk fat content decreased from 3.55 to 3.39% for CON but was not different for YEAST (3.51 to 3.47%; interaction $P = 0.05$). Overall, the live yeast supplement did not impact DMI or the yields of milk and milk components. Results demonstrate that a live yeast supplement may help prevent milk fat depression during transition to a diet with increased ruminal starch fermentability.

Key Words: live yeast, starch, milk fat

10 Manufacturing heat-stable whey protein isolate by clarification. G. Subbiah Prabhakaran*[1], J. A. Lucey[1,2], and M. Molitor[2], [1]*University of Wisconsin, Madison, Madison, WI*, [2]*Wisconsin Centre for Dairy Research*, *Madison, WI*.

Residual fat (RF) in whey protein isolate (WPI) is detrimental for storage flavor and functional applications. Objective of this study was to remove RF without utilizing microfiltration. We believe that by demineralizing whey via ultrafiltration (UF), phospholipoprotein (PLP) fractions can be precipitated along with denatured proteins and removed by centrifugation. Ca is also removed which improves heat stability. By removing RF and hence reducing oxidation of PLPs, we expect the resultant WPI to have a longer shelf life. Demineralization was achieved by acidifying liquid WPC-34 to pH 4.5 using HCl and ultrafiltering it along with extensive diafiltration. Benchtop trials (n = 4, $P < 0.05$) studied effects of pH (4.1, 4.3, 4.5, 4.7, 4.9, 5.2), protein concentration (1, 2, 3, 5, 7, 9% wb) and conductivity (300, 500, 750, 1000, 1500 mS/cm^2) of demineralized UF retentate (UF-r) on precipitation of RF. The UF-r was adjusted to various combinations of pH, protein concentrations (PC) and conductivities and centrifuged on a bench scale at 7500 × *g* for 10 min. Sedimentation of RF was estimated by measuring turbidity of supernatants. RF precipitation was highest at PC $\leq$3% because of reduced viscosity. At pH 4.5–4.7, precipitation was maximum showing that denatured protein and PLPs coagulate at pH values close to isoelectric point of denatured proteins. Reducing conductivity, increased precipitation of RF indicating that more coagulation occurs with reduced charge screening. SDS-PAGE analysis indicated there was sedimentation of PLPs, caseins and denatured proteins while native whey proteins remained soluble. Thus, isoelectric precipitation was effective in removing RF from sweet whey. Results were used to scale up the process. 200 gal of sweet whey was demineralized and clarified using a pilot-scale desludging clarifier and spray dried to produce WPI powder (91% protein, 2.6% fat db). Test for heat stability showed that 98% protein remained soluble when a 5% protein solution was heated treated at 80°C for 30 min. Future work will compare functional, flavor and storage properties of this WPI with some commercially available WPIs.

Key Words: whey protein isolate, isoelectric precipitation, clarification

11 Application of lactose oxidase to control *Pseudomonas* spp. and delay age gelation in UHT milk. V. K. Rivera Flores*, T. A. DeMarsh, and S. D. Alcaine, *Cornell University*, *Ithaca, NY*.

Shelf-stable milk is consumed worldwide, and this market is expected to continue growing. One quality challenge for UHT milk shelf life is age gelation, which can be caused by bacterial heat-stable proteases (HSP) synthesized during the raw milk storage period before heat processing. Some *Pseudomonas* spp. are HSP producers and their ability to grow well at refrigeration temperature make them important spoilage organisms to control for UHT processors. Previous work from our group showed that lactose oxidase (LO), a commercial enzyme that produces hydrogen peroxide and lactobionic acid from lactose, can control bacterial growth in raw milk. In this research, we investigated the ability of LO to control HSP producer outgrowth and thus prevent, or delay, age gelation in UHT milk. Six strains of *Pseudomonas* spp. were selected based on their ability to synthesize HSP and used as a cocktail to inoculate both raw and sterile milk at a level of 1×10^5 cfu/mL. Samples were treated with and without LO, incubated for 3 or 4 d at 6°C, and monitored for cell count and pH. A heat treatment was applied after the incubation period, and particle size analysis and visual inspection were used to monitor gelation from HSP activity. Coagulation assessment -analyzed using Tukey's HSD test- showed that in sterile milk, a LO treatment [0.12 g/L] was significantly different from the control ($P < 0.05$). In raw milk, however, a LO treatment of 0.24 g/L was needed to prevent gelation. The test was scaled up to 18.9 L pilot plant batches of raw milk, which were challenged with the *Pseudomonas* spp. cocktail, and treated with LO [0.24 g/L] for 3 d. Batches were then processed with a MicroThermics UHT unit, and bottles monitored for gelation at room temperature. Significant difference in particle size between the sample treated with LO and the control was observed as early as one month after processing ($P < 0.05$). These results demonstrate that LO can be used to prevent age gelation in UHT milk induced by HSP-producing *Pseudomonas* spp., representing an opportunity to improve quality and reduce post-production losses in the shelf-stable milk market sector.

Key Words: lactose oxidase, age gelation, UHT milk

12 Improving the extraction of dairy phospholipids by the combined effect of ultrasound pretreatment and switchable solvents. K. Rathnakumar*[1], J. Ortega-Anaya[2], R. Jimenez-Flores[2], J. Reineke[1], and S. I. Martínez-Monteagudo[1], [1]*South Dakota State University*, *Brookings, SD*, [2]*The Ohio State University*, *Columbus, OH*.

In the last decade, the consumption of phospholipids (PLs), a class of polar lipids, has been associated with important health benefits. Dairy by-products are an abundant source of PLs with potential for extraction and isolation. In this work, we report improvements in the extraction efficiency of PLs from Beta-serum, a dairy by-product, by the application of ultrasound (USD) before the extraction with switchable hydrophilicity solvent (SHS), these solvents can switch from hydrophobic to hydrophilic form by inserting the CO_2.The ultrasound pretreatment consisted of the application of 3 levels of acoustic intensity (either 134.15 ± 1.20, 274.91 ± 2.47, or 385.67 ± 3.47 W/m^{-2}) for 4 min. After the pretreatment, the extraction of the lipid fraction was performed using a tertiary amine (*N*,*N*-dimethylcyclohexylamine, CyNMe2) as a switchable hydrophilicity solvent (SHS) at 3 different sample to SHS ratios (1/3, 1/6 and 1/12). The PLs were recovered by solid phase-microextraction, and the individual PLs were quantified by HPLC with charged aerosol detector. The effect of acoustic intensity (385.67 ± 3.47 W/m^{-2}) followed by SHS extraction using 1/12 ratio recovered 69.07 ± 0.11% of PLs, while only 7.57 ± 0.59% of the PLs were recovered without the ultrasound pretreatment. The recovered fraction of PLs mainly comprised of phosphatidylinositol (32%), phosphatidylethanolamine (30%), and sphingomyelin (37%) found higher compared with untreated samples. Scanning electron images, particle size, and gel electrophoresis revealed great disruption of the protein matrix that may release the PLs into the aqueous medium. The application of ultrasound prior the SHS extraction remarkably improved the recovery efficiency of PLs. The proposed method improves the extraction of dairy PLs and may help to expand the utilization of thereof.

Key Words: switchable solvent, ultrasound, phospholipids

13 Effect of cold plasma treatment on the functional properties of MPC 85. S. Raghunath*, T. Schoenfuss, K. Mallikarjunan, and G. Annor, *University of Minnesota-Twin Cities*, *Saint Paul, MN*.

Milk protein concentrate (MPC) is a high-quality protein found in milk and a complete protein consisting of both casein and whey. MPC is known to have poor solubility and flowability due to physicochemical interactions. The cold plasma (CP) treatment is known to modify protein and thereby alter its functionality. The scope of this study is to improve functional characteristics of MPC after cold plasma treatment and understand mechanisms involved. In this study, MPC 85 powder was directly subjected to various treatment duration (15 min to 60 min) with cold plasma at 25°C, 120 W radio frequency (RF) power, and flowrates of 10 and 25 cm^3/min of argon and CO_2. The samples (treated and untreated) were then subjected to various physiochemical tests including flowability, water activity, solubility, moisture content, dry mass, foaming capacity, stability, pH, wettability, zeta potential, water binding capacity and solubility (at 50, 22 and 5°C) in triplicates. The statistical analysis was done using ANOVA, and the significant differences were calculated ($P < 0.05$) using Tukey HSD. Flow index significantly increased by 50% at 45 min. Foaming capacity and stability had a 63% (157.33 to 257.33 mL/g) and 64% (52.54 to 86.11%) increase till 30 min and then had a drop to 22% (192.00 mL/g) at 60 min whereas the stability remained constant. The pH remained constant in the range of 6.8 – 7.00. There was an 80% decrease (93s to 18.67s) in wettability and 38% (45.16 to 62%) increase in water binding capacity after 45 min. Zeta potential did not follow a particular trend. The dry mass remained constant, but the moisture content decreased to 77% (9 to 2). The solubility increased significantly after CP treatment but the maximum increase was observed at different time points for each temperature. The solubility at 50 and 5°C had an increase of 16% and 97%, respectively. The flowability, wettability, foaming capacity and stability, water binding capacity improved following the CP treatment due to the change in protein structure. Results proved that the samples showed better functionality in comparison to untreated samples. RF cold plasma could provide an alternative non-thermal processing approach to modify functional properties of MPC 85 and can be tailored for specific applications.

Key Words: cold plasma, functionality, milk protein concentrate

14 Shelf stability of milk protein gels created by pressure-assisted enzymatic gelation.

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Recent studies showed that high-pressure processing (HPP) provides exciting opportunities for structure formation in high concentration protein foods, with minimal impact on their overall nutritional and sensory properties. In this work, a new concept of pressure-assisted enzymatic gelation of milk protein concentrates (MPC) was applied, with the goal of further enhancing the structure and stability of pressure-induced milk protein gels, facilitating their use for the manufacture of novel dairy products. MPC powder was reconstituted to form a 12.5% (wt/wt) protein solution. Calf chymosin (45 IMCU/1000g milk) was added to the samples. Immediately after chymosin addition, the samples were treated with HPP at 600 MPa at 5°C for 3 min, followed by a shelf life study of 28 d at 4 ± 0.2°C. Textural analysis and water holding capacity measurements were carried out on d 0, 7, 14, 21, and 28. The processing trials and measurements were conducted in triplicate. Statistical analyses were performed by ANOVA at a 95% confidence level. Pressurization of MPCs led to extensive protein aggregation and gel formation, in a much shorter time (3min) compared with conventional enzymatic coagulation (around 30 min). The gel hardness of MPCs with added chymosin was 297 ± 61g at the beginning of storage and remained at 410 ± 19 g after 7 d. The water holding capacity remained at 91 ± 1% during refrigerated storage. Without chymosin, HPP-created MPC gels had a lower gel hardness value, of 227 ± 54g, which decreased by 5.9% to 213 ± 10 g during refrigerated storage. However, the water holding capacity remained at 100% during 28 d of refrigerated storage. These results clearly show that enzymatic coagulation under pressure can create gel structures that are stable during 28 d of refrigeration. These findings demonstrate that controlled, fast structural modification of high concentration protein systems can be obtained by pressure-assisted enzymatic treatment. Overall, this study provides insights into the possibility of using HPP for the development of milk-protein based products with novel structures and extended shelf life.

Key Words: high-pressure processing (HPP), milk protein gels, shelf stability

15 A method to diagnose mid-infrared milk analyzer prediction equation performance.

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Mid-infrared (MIR) milk analyzers are used for milk payment and product testing. Our objective was to determine if a modified milk calibration sample set could be used to diagnose and identify weaknesses in both partial least squares (PLS) based and traditional fixed-filter based predictions of milk component concentration. The modified milk calibration set (14 samples with a wide range of fat, protein, lactose and urea), is formulated in an orthogonal design and all-lab mean reference chemistry that, allows the identification of specific weaknesses in MIR prediction equations that are due to incorrect compensation for variation in the background milk matrix effects of fat, protein, and lactose concentration. In the case of traditional fixed-filter prediction models, the calibration equations can be adjusted based on the results of analysis of the modified milk set to improve instrument accuracy, while in the case of PLS models, specific model weaknesses can be identified and pointed out to the PLS model developer. For traditional filter models that predict fat, protein, and lactose, the sensitivity of predicted values to a mismatch of the intercorrection factor settings with the instrument optic system characteristics caused the standard deviation of the difference (SDD) between instrument prediction and reference chemistry to be larger (e.g., SDD of 0.004 vs 0.021 for fat when intercorrection factor for protein on fat B differs by 0.03), with systematic under or over estimation of the component being predicted at the ends of the concentration range of the interfering milk component. For PLS models, the inability of a PLS model to cancel out the background matrix variation effects of fat, protein, and lactose concentration on the parameter being predicted can be clearly identified and quantified. Based on this diagnostic data that can be produced by analysis of the modified milk samples, the population of milk sample spectra that need to be added to the PLS modeling population to improve the prediction accuracy of a PLS model measuring major milk components, or for prediction models for minor milk components (e.g., milk urea nitrogen or fatty acids) can be determined.

Key Words: mid-infrared, intercorrection factor, partial least squares models

16 Impact of milk protein type and concentration on the composition, physical, and sensory properties of low-fat, high-protein ice cream.

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The market for frozen desserts with added protein has grown in the last decade. Milk proteins are important in the development of ice cream structure. The objective of this study was to determine the effect of liquid dairy proteins on the composition, physical, and sensory properties of low-fat (about 4%), high-protein ice cream. Ice creams were formulated in duplicate with liquid micellar casein concentrate (MCC), milk protein concentrate (MPC), or whey protein isolate (WPI) to contain 3, 6, or 9% crude protein (CP, total nitrogen x 6.38) for each protein type. The composition, color, particle size, and viscosity of ice cream mixes were determined. Mix was frozen on a continuous freezer, and the overrun and particle size of frozen ice creams were determined. The color, meltdown rate, and trained panel flavor and texture attributes were determined for ice creams following 0, 1, 2, and 3 mo storage. Ice cream made with WPI had higher mix viscosity ($P < 0.05$), and lower overrun than MCC or MPC ice cream ($P < 0.05$). WPI ice cream also had a larger mean particle size and wider particle size distribution before and after freezing than MCC and MPC ice creams ($P < 0.05$). Vanilla flavor was highest in MCC ice creams ($P < 0.05$) followed by MPC and then WPI ice creams. Ice creams with lower protein had higher vanilla flavor (3 > 6 > 9% CP) ($P < 0.05$). Vanilla flavor also declined over time for all protein types and concentrations ($P < 0.05$). Astringency increased with increasing CP, and WPI ice creams were higher in astringency than MCC or MPC ice creams ($P < 0.05$). Cardboard flavor developed in ice creams over time, and this flavor intensity was higher in higher CP ice creams ($P < 0.05$). Firmness, denseness, mouth coating, and melted viscosity increased with increasing CP ($P < 0.05$), whereas crumbliness had an inverse relationship with CP. MCC ice cream was lower in mouth coating and melted viscosity than MPC and WPI ice creams ($P < 0.05$). Over time, a decrease in crumbliness and mouth coating was observed in ice creams while denseness and smoothness increased

($P < 0.05$). This research elucidates the impact of milk protein source and protein concentration in ice cream on structural and sensory properties.

Key Words: ice cream, high protein, low fat

17 Decarboxylation of hydroxycinnamic acids by lactic acid bacteria strains isolated from dairy products.

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Food flavor and aroma are significantly impacted by the presence of 4-vinylphenols (4VPs), volatile compounds with very low-perception thresholds produced by decarboxylation of hydroxycinnamic acids (HCAs) ubiquitous in nature. 4VPs can be found in dairy products such as cheese and yogurt, and when in low concentrations, they contribute positively to the flavor by adding complexity and uniqueness. In HCA-rich foods subjected to bacterial fermentation, it is key to select strains that will produce the desired sensory properties, highlighting the need for screening lactic acid bacteria (LAB) for decarboxylating ability. The decarboxylating activity of LAB strains from the Ohio State University–Parker Endowed Chair collection (137 strains) with potential to produce phenolic acid decarboxylase (22 strains) was evaluated after incubation with HCAs for 36 h at 32°C. Decarboxylation was monitored using a high-throughput spectrophotometric method based on hypsochromic shifts when HCAs are transformed into 4VPs. Spectrophotometric results were confirmed by HPLC-DAD-MS analyses, looking for longer retention times and shorter $\lambda_{230\text{–}500\ \max}$ than their precursor HCA, and characteristic m/z. *Enterococcus mundtii*, *Lactobacillus plantarum* and *Pediococcus pentosaceus* were capable of decarboxylating *p*-coumaric, caffeic and ferulic acids producing their 4VP derivatives. Seven other strains were only capable of biotransforming *p*-coumaric and caffeic acid, 1 was able to decarboxylate only caffeic acid and 1 was able to decarboxylate only *p*-coumaric acid, while 10 strains were not able to biotransform any HCA. No strain in this study was capable of decarboxylating sinapic acid. *p*-Coumaric acid had the highest biotransformation efficiency, followed by caffeic acid and lastly ferulic acid. This is the first study showing decarboxylating activity by the *E. mundtii* strain. This work can help improve LAB strain selection for food applications, improving the sensorial characteristics of fermented dairy products such as cheese and yogurt, especially the ones formulated with HCA-rich fruit and vegetable extracts.

Key Words: microbial biotransformation, fermented food, flavor compound

18 Radiofrequency-assisted thermal process validation of dairy powder considering the effect of storage time.

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Salmonella persistence in dairy powders has caused several multistate foodborne outbreaks. Therefore, effective antimicrobial treatments need to be identified and validated to ensure the safety of dairy powders. Because storage time may influence the thermal resistance of *Salmonella* in dairy powders, this factor needs to be included in the process validation. The objectives of this study were to evaluate the effect of storage time on the thermal resistance of *Salmonella* in dairy powders; evaluate the suitability of *Enterococcus faecium* NRRL B-2354 as a non-pathogenic surrogate for *Salmonella* during thermal processing; and validate radiofrequency (RF)-assisted thermal processing as a suitable pasteurization process to inactivate *Salmonella* in dairy powders. Nonfat dry milk and whole milk powder were inoculated with a 5-strain *Salmonella* cocktail or *E. faecium* NRRL B-2354 and equilibrated to a low water activity (a_w) level of 0.10 representing an extreme worst-case scenario (typical a_w is 0.20). The samples were stored for 30, 60, and 90 d and decimal reduction values (*D*-values) were determined at 85°C. *D*-values were also determined at 90 and 95°C at 30 d of storage to identify the adequate thermal processing time and temperature combination for RF-assisted thermal processing. A custom-designed thermal death sandwich was used to conduct the isothermal treatments. Based on the determined processing conditions, RF heating of dairy powders was conducted in a 6-kW, 27.12-MHz pilot scale parallel plate RF heating system to achieve the target temperature and then transferred to a convective hot air oven for specific holding time. Results showed that storage time did not significantly ($P > 0.05$) affect the thermal resistance of *Salmonella* in dairy powders. *E. faecium* was a suitable surrogate for *Salmonella* due to its significantly ($P < 0.05$) higher thermal resistance (*D*-value) and similar inactivation kinetics during thermal treatment of dairy powders. RF studies are currently being conducted at 85, 90, and 95°C to validate at least a 5-log reduction of *Salmonella* in dairy powders.

Key Words: surrogate, thermal death time, dielectric heating

19 The effect of pre-, pro-, and symbiotic supplementation in milk to pre-weaned Holstein heifers on body weight gain and health outcomes. P. Lucey*[1], I. Lean[2], S. Aly[1], H. Golder[2], and H. Rossow[1], [1]*University of California-Davis*, *Davis, CA*, [2]*Scibus*, *Camden, NSW, Australia*.

The objective of this trial was to evaluate the effectiveness of pro-, pre-, and symbiotic supplementation on average daily weight gain (ADG), diarrhea and odds of pneumonia treatment with an antibiotic in pre-weaned dairy heifer calves. A total of 1,801 healthy Holstein heifer calves from a large California dairy were enrolled at 4 – 12 h of age until weaning at approximately 60 d of age. Calves were block-randomized on a repeating random order of all possible orderings of the 4 treatments: 1) control, 2) enzymatically hydrolyzed yeast product (pre), 3) *Bacillus subtilis* (pro), 4) combination of both products (symbiotic), which were administered in milk twice daily from enrollment until weaning. Serum total protein at enrollment and BW at 7, 42 and 56 d of age were measured. Fecal consistency was assessed daily on a scoring system of 1 to 3, for the entire pre-weaned period. Linear regression showed that symbiotic treated calves had 20 g increased ADG compared with control calves for overall BW gain (7 – 56 d) ($P = 0.042$). For late BW gain (42 – 56 d), prebiotic treated calves had 84 g increased ADG ($P = 0.007$) and symbiotic treated calves had 79 g increased ADG ($P = 0.012$) compared with control calves. Linear regression showed no difference in the length of the first diarrhea episode per calf by treatment. Cox regression showed no difference in hazard of diarrhea compared with control calves. Logistic regression showed no difference in the odds of calves experiencing pneumonia. Although no effects were seen on diarrhea length or pneumonia incidence, superior BW gain in the late pre-weaned period may increase health outcomes of calves across the weaning process.

Key Words: calf, probiotic, prebiotic

20 Metabolomic profile differs between Holstein strains at grazing conditions during early and mid lactation. E. Jorge-Smeding*[1], D. Armand-Ugon[2], A. Mendoza[2], M. Carriquiry[1], and A. Astessiano[1], [1]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República*, *Montevideo, Uruguay*, [2]*Programa de Producción de LecheUnidad de Lechería, Estación Experimental INIA La Estanzuela, Instituto Nacional de Investigación Agropecuaria*, *Colonia, Uruguay*.

The objective of this study was to evaluate the effect of Holstein strain on plasma metabolome to understand physiological adaptation at grazing of Holstein cows of 2 genetic origins: American (HFA, n = 8; 588 kg live weight, 2.60 BCS) and New Zealand (HFNZ, n = 8, 524 kg live weight, 2.50 BCS) during early and mid-lactation. Multiparous cows were used. Cows were fed 64% pastures, 31% of concentrate and 5% on average. Cows produced 33.0 vs. 30.4 ± 2.0 kg/d of milk for HFA and HFNZ respectively ($P = 0.02$), without differences between genetic strains in body condition score ($P = 0.10$). Plasma were collected at 21 and 180 d in milk and analyzed using a targeted metabolomic approach through a gas chromatography/time-of-flight mass spectrometry method. A total of 172 metabolites were quantified. According to principal component analysis, data were clustered in 2 groups corresponding to DIM and a partial least square discriminant analysis classification model was obtained ($R^2 = 0.95$; $Q^2 = 0.40$) being oxoproline, *p*-tolyl glucuronide, 5-hydroxynorvaline, erythritol and isoleucine the 5 metabolites with the highest score of variable importance in the projection (VIP score >2.0 in all cases). Univariate analysis (false discovery rate-corrected ANOVA) showed 68 metabolites differing (adj-$P < 0.05$) between strains and 5 that tended (adj-$P < 0.10$) to be affected by the interaction between strain and DIM, but none of the metabolites were affected by DIM. Most of these differing metabolites belonged to amino acid metabolism (n = 26), fatty acids (n = 11), kidney deficiency pathways (n = 4), bile acids (n = 3) and the others composed single metabolite classes. There was not a clear trend on the effect of strain for most of the metabolite families as — on average— within each group (AA, fatty acid, kidney deficiency pathways, bile acids), half of the metabolites had a fold change >1.0 and half <1.0 for HFNZ compared with HFA cows. However, with the exception of linoleic and stearic acid, all fatty acids were increased in HFNZ compared with HFA cows (e.g., palmitoleic, arachidonic acids, adj-$P < 0.05$, fold change >1.0 in all cases). The effect of genetic strain on metabolic adaptations to pastoral constrains seems to rely, at least in part, on nitrogen metabolism.

Key Words: Holstein strains, grazing, metabolomics

21 Simultaneous minimization of diet costs and phosphorus excretion on dairy farms. A. F. White* and L. E. Moraes, *The Ohio State University*, *Columbus, OH*.

The study objective was to develop a mathematical programming model capable of simultaneously optimizing diets with different weights for least cost (LC) and least on farm P-balance (LP). The ration ingredients, herd composition, and animal categories were designed to represent the Jersey herd of the Ohio State University Waterman Dairy Center. The model constraints were set to meet the current recommendations of the NRC (2001), and additional constraints based on industry recommended practices were specified to limit the inclusion of specific feeds in the diet. Using OpenSolver (v.2.9.0, opensolver.org), 3 objective functions were individually optimized to (1) minimize the diet cost; (2) minimize the excess of P balance in the system, computed as the difference between P excreted and P uptake by forage production on-farm; and (3) minimize the weighted deviations from the values of the 2 previously optimized objective functions. A grid of 100 potential weights were used that varied the size of the effect of each deviation from the given optimization model on the current optimized objective. Total daily feed cost per kg of milk for LC and LP were \$0.21 and \$0.25 (19% increase with respect to the LC diet), respectively; daily farm P balance per kg milk was 0.9g and 0.3g (67% decrease). Thus, at the extreme points of the weight grid, a substantial decrease in P balance was obtained; however, at a substantial increase in diet costs. The trade-offs between the 2 goals set by the weighting scheme, however, allowed the identification of a set of diets that all met the NRC (2001) requirements while having different costs and determining different P balances on the farm. For example, the weighted goal programming model identified a diet that allowed a 37% reduction of P balance occurring with a 0.4% increase in cost (with respect to LC). These initial reductions in P were generally accompanied by increased forage fed, increase of on-farm corn silage production, and increased purchase of hay. These optimizations suggest a potential use of weighted goal programming as a technique to identify diets that allow a reduction in on-farm P balance with limited effect on whole-farm feed cost.

22 Predictive models for early diagnosis of metritis and clinical cure of dairy cows using behavioral parameters. V. R. Merenda*[1] and R. C. Chebel[1,2], [1]*Department of Large Animal Clinical Sciences, University of Florida*, *Gainesville, FL*, [2]*Department of Animal Sciences, Institute of Food and Agricultural Sciences*, *Gainesville, FL*.

Our objectives were to predict the occurrence of metritis and cure from metritis. Holstein animals (n = 564) were fitted with an automated device 21 d prepartum. Cows were examined for metritis (fetid, watery, red/brown uterine discharge) and, at diagnosis (d 0), were paired by parity and severity of metritis [metritis vs. puerperal (rectal temperature ≥39.5°C)] and were randomly selected to receive ampicillin trihydrate or ceftiofur crystalline free acid. Cows that were not diagnosed with any diseases (n = 374) were enrolled in the study. Cure from metritis was defined as the absence of metritic discharge and rectal temperature <39.5°C, 11 d after diagnosis. Rumination, resting, feeding and activity time from 10 d prepartum until 10 d after metritis diagnosis were used. In all models, farm, lactation, retained placenta, vaginal laceration score, stillbirth, male, twins, dystocia, rumination, resting, feeding, and activity time were included. In addition, BCS prepartum and at calving were included in the models to predict the occurrence of metritis, and severity of metritis and BCS at diagnosis were included in the models to predict the probability of cure. The area under the curve (AUC) according to the receiver operator characteristic for the model predicting metritis at 1 [AUC = 0.74, 95% confidence interval (CI) = 0.70, 0.78] and 2 (AUC = 0.78, 95% CI = 0.74, 0.81) DIM were ($P < 0.01$) acceptable. The models predicting puerperal metritis at 1 (AUC = 0.81, 95% CI = 0.77, 0.84) and 2 (0.82; 95% CI = 0.78, 0.85) DIM were ($P < 0.01$) excellent. The AUC of the models using behavioral changes peripartum to predict cure at d −1 (0.81; 95% CI = 0.74, 0.87) and 0 (0.81; 95% CI = 0.75, 0.87) relative to diagnosis were ($P < 0.01$) excellent, as was the AUC of the model using total behavior time on d 0 (AUC = 0.82, 95% CI = 0.75, 0.88; $P < 0.01$). This study demonstrates that behavioral changes peripartum allows for the early diagnosis of metritis and whether cows will be cured from metritis, allowing for the implementation of preventive strategies and earlier decision making regarding metritis treatment.

Key Words: predictive model, metritis, cure

23 Effect of a commercial active dry yeast (CNCM I-1079) on productive and metabolic measures during the periparturient transition. M. R. Steelreath*[1], H. C. Hung[1], R. L. Hiltz[1], M. N. Degenshein[1], A. Aguilar[2], H. Nielsen[2], A. H. Laarman[3], and P. Rezamand[1], [1]*Department of Animal and Veterinary Science, University of Idaho*, *Moscow, ID*, [2]*Lallemand Animal Nutrition*, *Milwaukee, WI*, [3]*Agricultural Food and Nutritional Science, University of Alberta*, *Edmonton, AB, Canada*.

The periparturient period is a metabolically demanding time for dairy animals because of the increased nutrient requirements for milk yield. The objective of this study was to investigate the effect of feeding a commercial active dry yeast (ADY) in dairy cows on productive and metabolic measures during the periparturient period. Primiparous (n = 33) and multiparous (n = 35) cows were fed a close-up TMR before calving and a lactation TMR postpartum. Three weeks before expected calving time, animals were blocked by parity and BW and then randomly assigned to either control group (CTRL; n = 34) or treatment (ADY; n = 34). The ADY animals received a top-dressed ADY (*S.c. Boulardii*, CNCM-1079) fed daily at 12.5 g per head (10×10^9 cfu). Blood samples were collected weekly. Milk yield, feed intake, and milk composition data were collected. Plasma/serum samples were analyzed for glucose, nonesterified fatty acid (NEFA), β-hydroxybutyrate (BHB), and haptoglobin (Hpt). Colostrum samples collected within the first 6–10 h were analyzed for somatic cell count (SCC) and IgG, IgA, IgM concentrations. Data were analyzed using PROC MIXED in SAS with time as a repeated measure. Model included time, parity, treatment, and their interactions. Significance was declared as $P \leq 0.05$ and tendency at $P < 0.1$. The ADY animals produced more milk (39.3 vs 37.2 ± 2.02 kg/d; $P = 0.02$), tended to produce more energy-corrected milk (ECM; 35.5 vs 34.2 ± 1.44 kg/d; $P = 0.07$), and had a better feed efficiency (ECM/DMI; $P = 0.06$). There was a treatment × parity × time effect on milk protein percent; the multiparous ADY animals had a greater protein percent wk 2 postpartum ($P < 0.01$). There was not a difference in plasma glucose, serum NEFA, serum BHB, or colostrum IgG, IgA, and IgM by main effects or their interactions. Multiparous animals had a greater colostrum IgA (1011 vs. 749 ± 38 mg/dL) but a lower SCC (926 vs. 1578 × 1000/mL). ADY animals had a greater serum Hpt ($P = 0.03$) while Hpt concentration peaked for all animals wk 1 postpartum. Research is needed to understand the effects of this ADY on metabolism.

Key Words: active dry yeast, periparturient cow, blood metabolites

24 Identifying factors associated with lameness and its impact on productivity in automated milking herds. R. D. Matson*[1], M. T. M. King[1], T. F. Duffield[2], D. E. Santschi[3], K. Orsel[4], E. A. Pajor[4], G. B. Penner[5], T. Mutsvangwa[5], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph*, *Guelph, ON, Canada*, [2]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*, [3]*Lactanet*, *Sainte-Anne-de-Bellevue, QC, Canada*, [4]*Faculty of Veterinary Medicine, University of Calgary*, *Calgary, AB, Canada*, [5]*Department of Animal and Poultry Science, University of Saskatchewan*, *Saskatoon, SK, Canada*.

Voluntary milking is critical for success in automated milking systems (AMS); impaired gait (lameness) may negatively affect the ability and desire for cows to milk voluntarily. The objective of this study was to assess the impact lameness has on the productivity of AMS herds and identify factors associated with lameness. From April to September 2019, 76 robot herds were visited, and data on barn design and farm management practices were collected. Data from AMS units were collected, along with milk recording data for the 6 mo period before farm visits. Farms averaged 99 ± 73 lactating cows, 2.3 ± 1.4 robot units/farm, 43.9 ± 9.0 cows/robot, 36.7 ± 4.7 kg/d of milk, a milking frequency of 3.0 ± 0.4×/d, and a herd-average SCC of 198.3 ± 88.1 (×1,000) cells/mL. Thirty percent (minimum of 30 cows/farm) were scored for body condition (BCS 1 = underconditioned to 5 = over conditioned) and gait (1 = sound to 5 = lame; with clinically lame ≥ 3: 28.6 ± 11.7%; and severely lame ≥ 4: 3.0 ± 3.2%). Univariable models were used to screen independent variables (as fixed effects) in mixed-effect linear regression models and variables with $P < 0.25$ were offered to multivariable models. Clinical lameness was 10.2 percentage points (p.p.) less prevalent on farms with sand bedding ($P < 0.01$) and tended to be 2.8 p.p. lesser for each additional time stalls were raked/d ($P = 0.07$) and 5.7 p.p. lesser for farms that built new barns vs. retrofitting existing barns ($P = 0.07$). Herd average milk yield/cow decreased with greater prevalence of clinical (−0.1kg/d for 1 p.p. increase; $P = 0.01$) and severe lameness (−0.8 kg/d with doubling of prevalence from 3 to 6%; $P < 0.01$). Milk yield/robot decreased with a greater prevalence of clinical lameness (−7.1 kg/d for 1 p.p. increase; $P = 0.01$). Lesser milking frequency was associated with a greater proportion of over-conditioned cows ($P = 0.04$). SCC was associated with a greater proportion of clinically lame ($P < 0.01$) and under-conditioned cows ($P = 0.05$). Overall, this study demonstrates that productivity and milk quality in AMS herds are optimized by maintaining mobility and body condition of cows.

Key Words: automated milking system, lameness, herd management

25 Heat stress abatement for late-gestation dairy heifers improves thermoregulation and productivity. B. D. Davidson*, B. Dado-Senn, N. Rosa Padilla, T. F. Fabris, L. T. Casarotto, V. G. Ouellet, G. E. Dahl, and J. Laporta, *Department of Animal Sciences, University of Florida*, *Gainesville, FL*.

Multiparous cows are negatively impacted by heat stress when dry, however, the impact of heat stress on pregnant heifers has received less attention. Our goal was to determine if late gestational heat stress abatement improves thermoregulatory responses and milk production of nulliparous heifers. Pregnant heifers were randomly assigned to either heat stress (HT, shade; n = 16) or cooling (CL, shade, soakers, and fans; n = 15) environments during the last 60 d of pregnancy (~8 weeks). Rectal temperatures (thermometer), respiration rates (flank movements/min), sweating rates (SR; VapoMeter), and skin temperatures (ST; infrared thermometer) were measured thrice weekly from enrollment to calving. Vaginal temperatures (i-button intra-vaginal device) were measured every 10 min for 7 consecutive days at wk 1, 3, 5, and 7 relative to enrollment. Daily thermoregulatory patterns were assessed by SR and ST measured every 4 h over a 36-h time interval at wk 3, 5, and 7. Milk yield was recorded twice daily for 15 weeks. Data were analyzed by PROC MIXED (repeated measures) with treatment, hour, week, and all possible interactions as fixed effects. Cool heifers had lower (all $P \leq 0.01$) rectal temperatures (38.7 vs. 38.9°C), respiration rates (46.3 vs. 59.6 bpm), and ST (34.8 vs. 35.3°C) relative to HT heifers. Sweating rates were lower in the CL vs. HT heifers from wk 2 to 8 ($P < 0.0001$). Vaginal temperatures were lower ($P = 0.002$) in CL relative to HT heifers during wk 1, 3, and 7, particularly between 1000 h to 1400 h and 2200 h to 0500 h. When measured continuously over a 36-h time interval ST and SR were lower ($P \leq 0.006$) in CL heifers for all weeks; notably, CL heifer ST was reduced overnight and SR was reduced during the daytime. Cooled heifers had higher milk yield (3.7 kg/d, $P = 0.05$) when compared with HT heifers during all weeks except wk 2 and 5, in which there was a tendency ($P \leq 0.08$) for higher milk production in CL heifers. Similar to multiparous cows, our data indicate that actively cooling heifers in late pregnancy is effective in improving thermoregulation and production.

Key Words: cooling, heifer, milk yield

26 The effects of feeding pelleted dried distillers grains with different concentrations of forage on milk production, nutrient digestibility, passage rate, rumen characteristics, and chewing behavior of lactating Jersey dairy cows. K. C. Krogstad*[1], K. J. Herrick[2], and P. J. Kononoff[1], [1]*Department of Animal Science, University of Nebraska-Lincoln*, *Lincoln, NE*, [2]*POET Nutrition*, *Sioux Falls, SD*.

Particle size or processing of feeds can influence chewing behavior, rumen characteristics, rumen passage rate, and digestibility. Changing particle size is usually achieved through grinding or chopping forages, but processing feed ingredients by pelleting them also changes particle size. Seven rumen cannulated lactating Jersey cows averaging 126 ± 33.3 (Avg ± SD) DIM and 462 ± 36.7 kg of BW were used in a crossover design. The treatments contained 15% DM of DDGS in either meal or pellet form along with a diet containing either 45% or 55% forage on a DM basis. The forages used were corn silage, alfalfa hay, and wheat straw. The factorial treatment arrangement was as follows; meal-form DDGS and low forage (LLF), pelleted DDGS and low forage (PLF), meal-form DDGS and high forage (LHF), and pelleted DDGS and high forage (PHF). Both dry matter intake and milk yield were unaffected by treatment ($P > 0.14$) averaging 19.8 ± 0.85 kg and 27.8 ± 1.22 kg. Fat yield was unaffected ($P > 0.32$) averaging 1.7 ± 0.06 kg, but protein yield was affected by the interaction of forage and DDGS ($P = 0.081$); Protein yield was 1.08, 1.05, 0.99, and 1.05 ± 0.035 kg for LLF, PLF, LHF, and PHF respectively. The digestibility of NDF and energy increased by 2.8 and 1.6 units, respectively ($P < 0.05$). Rumen mass and passage rate were not affected by treatment ($P > 0.21$) and averaged 10.0 ± 0.73 kg of DM and 2.7 ± 0.21%/h. Rumination time was increased from 417 min to 454 min by increasing the concentration of forage ($P = 0.08$). Rumen pH and ammonia also increased due to increasing forage concentration; pH increased from 5.86 to 5.92 ± 0.057 ($P = 0.04$) and rumen ammonia increased from 16.8 to 19.1 ± 3.22 mg/dL ($P = 0.002$). Outcomes confirm that increasing forage concentration increases rumen pH and rumination time. Our results also indicate that pelleting DDGS does not appear to affect milk production, rumen characteristics, or passage rate but may increase NDF and energy digestibility.

Key Words: dried distillers grains with solubles (DDGS), digestibility, rumen passage rate

27 Prepartum shifting light circadian rhythm disruptions cause insulin resistance in periparturient dairy cows. C. J. McCabe*, A. Suarez-Trujillo, T. M. Casey, and J. P. Boerman, *Department of Animal Sciences, Purdue University*, *West Lafayette, IN*.

Circadian clocks function to maintain homeostasis. Disruption of clocks by altering timing of external cues affects glucose metabolism in humans and rodents, with long-term disruption associated with development of metabolic diseases. The objective of this experiment was to analyze the effect of exposure to chronic light-dark cycle phase shifts from 5 weeks before expected calving (BEC) to parturition on glucose tolerance at 2 weeks prepartum and 1 week postpartum in dairy cows. Multiparous Holstein cows (n = 16) were exposed to 16 h of light and 8 h of dark (CON) or phase shifting (PS) of the start of the light cycle 6 h every 3 d beginning 35 d BEC. Following calving, both treatments were exposed to control lighting through 60 DIM. At 14 d BEC and 7 DIM, intravenous glucose tolerance tests were performed with a dose of 250 mg/kg BW of a 50% dextrose solution. Before dextrose administration, cows were fasted for one hour and baseline blood samples were taken at 15 and 5 min before administration to determine Revised Quantitative Insulin Sensitivity Check Index (RQUICKI). We observed no difference in insulin sensitivity between treatments at both physiological stages via RQUICKI ($P > 0.05$). Following dextrose administration, blood samples were taken at 14 time points over 3 h to quantify blood glucose, BHBA, insulin, and NEFA. Linear mixed model analysis indicated no effect of treatment on blood glucose by time point or area under the curve (AUC) for both stages ($P > 0.05$). At 14 BEC, there was no difference in baseline values, but insulin AUC was higher in PS compared with CON ($P = 0.05$; 4,302.8 vs. 2,386.3 ng/mL/180 min). At 7 DIM, there was no effect of treatment on glucose AUC ($P = 0.71$), but the difference in insulin AUC between PS and CON ($P = 0.03$; 1,052.9 vs. 697.1 ng/mL/180 min) was maintained. There was no effect of treatment on BHBA nor NEFA, but there was an interaction between treatment and time for NEFA at 7 DIM ($P = 0.007$). CON vs PS cows produced more milk through 60 DIM (42.6 vs. 40.3 kg/d; $P = 0.05$). Exposure to chronic light-dark PS in late gestation decreased insulin sensitivity in periparturient cows, which may influence milk production.

Key Words: insulin sensitivity, glucose tolerance test, circadian rhythm

ADSA Graduate Student Production PhD Oral Competition

28 Mitochondrial genome diversity and association of mitochondrial protein gene expression with energy metabolism in dairy cattle. J. Dorji*[1,2], C. J. Vander Jagt[2], J. B. Garner[3], L. C. Marett[2], B. A. Mason[2], C. M. Reich[2], C. P. Prowse-Wilkins[2,4], R. Xiang[2,4], P. N. Ho[2], J. Pryce[1,2], B. G. Cocks[1,2], A. J. Chamberlain[2], I. M. MacLeod[2], and H. D. Daetwyler[1,2], [1]*School of Applied Systems Biology, La Trobe University*, *Bundoora, Victoria, Australia*, [2]*Agriculture Victoria, AgriBio, Centre for AgriBioscience*, *Bundoora, Victoria, Australia*, [3]*Agriculture Victoria, Ellinbank Dairy Centre*, *Ellinbank, Victoria, Australia*, [4]*Faculty of Veterinary & Agricultural Science, University of Melbourne*, *Parkville, Australia*.

Mitochondria are primarily organelles for cellular energy metabolism and have a maternally inherited genome encoding 37 genes. Proteins from these genes interact with mitochondrial proteins (MP) encoded by nuclear genes to enable mitochondrial functions. Given the key role of mitochondria in energy production, mutations affecting the expression of MP genes could have flow-on effects on important traits in cattle. Our study had 3 aims: first to assess the diversity of the mitochondrial genome of modern dairy cattle breeds, second, to characterize MP gene expression across tissues within animals, and third, to correlate MP gene expression in blood with feed efficiency in dairy cattle. Mitochondrial genome diversities (nucleotide and haplotype) were estimated based on selected variant positions across the genome. Overall, there was a low diversity in the dairy breeds studied. Broadly, the modern dairy cattle (e.g., Holsteins) were predominantly T3 (~95%, with > 10 evident subgroups) and to a lesser extent T2 and T1 haplogroups. Gene expression in tissue as was quantified by RNA sequencing. We used differential expression and co-expression analyses of genes in 29 tissues from 2 cows. We found consistent overexpression in high energy demand tissues (e.g., heart). This suggests that MP gene expression might also differ between animals that differ within tissue energy demands. We, therefore, measured gene expression in blood samples of 2 groups (14 dairy cows each) divergent for feed efficiency to analyze the differential gene expression and co-expression networks. There were 395 genes differentially expressed (DE) between high and low feed efficiency groups, of which 55 were MP genes. Furthermore, DE genes were significantly enriched for oxidative phosphorylation (OxPhos), an important pathway that generates cellular energy. However, none of the DE MP genes was from the mitochondrial genome. The association between feed efficiency and expression of MP genes involved in the OxPhos pathway was also evident in a weighted gene co-expression network analysis ($r = 0.47$, $P = 0.01$). Altogether, our study suggests that there is low mitochondrial genomic diversity among popular dairy breeds and MP gene expression may be associated with variation in traits linked to mitochondrial function.

Key Words: mitogenome, gene expression, cattle

29 Bioactivity of the endocannabinoid arachidonoylethanolamide in cultured bovine endothelial cells. C. C. F. Walker* and L. M. Sordillo, *Michigan State University*, *East Lansing, MI*.

Vascular endothelial cells are crucial inflammatory-mediating cells susceptible to compromised barrier integrity during coliform mastitis. Endocannabinoid arachidonoylethanolamide (AEA) is a lipid mediator that can affect barrier integrity through modulation of network formation, proliferation, and viability in human and rodent endothelial cells during endotoxin challenge. We hypothesized that the endocannabinoid AEA will increase barrier integrity of primary cultured bovine aortic endothelial cells (BAEC) challenged with lipopolysaccharide (LPS). Cells were treated with varying AEA concentrations (10 n*M* to 5 μ*M*) for up to 12 h following pre-treatment with 25 ng/mL LPS. Endothelial barrier integrity was continuously assessed by recording electrical resistance using an electric cell-substrate impedance sensing system and reported resistance was normalized to a media control. Cell proliferation and viability were assessed using commercially available plate-based assays. All AEA treatments were compared with LPS control group and analyzed using the ANOVA procedure in SAS9.4. Physiological-relevant concentrations (<1 μ*M*) increased proliferation and viability of BAEC by up to 21.5 ± 2.283% and 19.37 ± 2.564%, respectively, at 1 and 2 h post-treatment ($P < 0.05$). Normalized electrical resistance was increased for 2 h after AEA treatment at 100 n*M* (0.105 ± 0.0137 Ω) and 500 n*M* (0.123 ± 0.015 Ω) ($P < 0.05$). No differences in resistance were noted for any other treatments <1 μ*M* ($P > 0.05$). Doses >1 μ*M* decreased electrical resistance, proliferation, and viability for up to 2 h post-treatment ($P < 0.05$). All treatment effects were lost at 6 or 12 h ($P > 0.05$). Physiological AEA concentrations of <1 μ*M* improve barrier integrity of BAEC when challenged with LPS, in contrast to the potential toxicity associated with super-physiological concentrations of >1 μ*M*. Ongoing projects are focused on the mechanistic effects of AEA on endothelial cell barrier integrity with the goal of reducing the severity of coliform mastitis.

Key Words: coliform mastitis, endocannabinoids, endothelial cells

30 Effect of feeding *Camelina sativa* cake on rumen microbiota and gene expression in follicular cells in dairy Italian Holstein Friesian heifers. F. Omodei Zorini*[1], M. Dell'Anno[1], G. Pennarossa[1], G. Morini[2], G. Ranzenigo[3], V. Caprarulo[1], L. Rossi[1], P. Cremonesi[4], B. Castiglioni[4], F. Biscarini[4], and G. Invernizzi[1], [1]*Department of Health, Animal Science and Food Safety 'Carlo Cantoni', University of Milan*, *Milan, Italy*, [2]*Department of Veterinary Medical Sciences, University of Parma*, *Parma, Italy*, [3]*DVM*, *Varese, Italy*, [4]*Institute for Biology and Biotechnology in Agriculture (IBBA), NRC*, *Lodi, Italy*.

Omega-3 PUFAs have unique role in several biological systems in mammals. However, its impact on rumen microbial environment could represent a critical point for both rumen welfare and its availability for the animal. The aim of the study was to evaluate the effects of the inclusion in the diet of a terrestrial vegetable and sustainable source of omega-3 PUFAs (*Camelina sativa* cake) in dairy heifers on rumen microbiota and gene expression of fertility markers in follicular cells. The trial was conducted at the tie stall dairy barn of the Experimental Farm of Animal Production Research and Teaching Centre of Lodi, University of Milan, Italy and lasted 56 d. Sixteen Italian Holstein Friesian heifers 12 mo old were divided in 2 homogeneous groups randomly allocated to 2 treatments: CAME ($n = 8$) receiving the basal diet supplemented with 800 g/head/day of camelina cake and CTR ($n = 8$) receiving the basal diet supplemented with an isonitrogenous and isoenergetic soybean-based premix. Basal diet consisted of a dry TMR composed by alfalfa hay, durum wheat middlings, sugar cane molasses, corn meal, soybean hulls, rice bran, sunflower meal and minerals (15.15 PG, 42.63 NDF, 19.43 starch on DM basis). Performances were recorded weekly, feed intake daily. Rumen and follicular content samples were collected at d 0, 28 and 56 of the trial. Follicular developmental competence was assessed by RT-PCR analysis. Performance data were analyzed by PROC MIXED of SAS for repeated measures. No differences were detected for live BW, FCR and BCS, but an interaction between diet and time was observed ($P \leq 0.05$) on DMI with higher values for CAME at d 34, 51, 52 and 55 compared with CTR (14.44 ± 0.93 kg vs. 10.47 ± 0.93 kg; 13.36 ± 0.93 kg vs. 10.81 ± 0.93 kg; 13.21 ± 0.93 kg vs. 10.86 ± 0.93 kg; 13.99 ± 0.93 kg vs. 11.15 ± 0.93 kg). Higher expression levels for *HAS2*, *GREM1*, *LHCGR* and *FSHR* genes were detected in CAME group compared with CTR, suggesting a positive effect of treatment diet. Rumen microbiota was influenced by dietary treatment both at 28 and 56 d showing significant α diversity values.

Key Words: camelina cake, fertility, rumen microbiota

31 One plus one is … three? Evidence for a compounding effect of long-chain fatty acids on peroxisome proliferator-activated receptor

activity. S. Busato* and M. Bionaz, *Oregon State University*, *Corvallis, OR*.

Peroxisome proliferator-activated receptors (PPAR) are transcription factors with known nutrigenomic response to fatty acids. In ruminants, PPAR control the expression of genes involved in lipid and glucose metabolism, anti-inflammatory response, and milk fat synthesis. While information on the in vitro potency to activate PPAR of some individual fatty acids exists, we were interested in exploring the activation of PPAR by a wider range of fatty acids and their combination. We hypothesized that the activation of PPAR with the combination of fatty acids is larger than the sum of the effect of individual fatty acids. We assessed in BFH-12 cells (immortalized bovine hepatocytes) the dose-response of 10 fatty acids common in bovine nutrition. PPAR activation was assessed by a gene reporter containing luciferase (3xPPAR response element) and normalized by renilla. Doses from 0 to 500 µM were applied using a HP D300e digital dispenser. Results were analyzed using GLM of SAS with dose as main effect and replicates (n = 4) as random. Response to each fatty acid was variable both in extent and in dose at maximum activation, with palmitic, stearic and dodecanoic acid showing the greatest impact on PPAR activation (3.5-fold, 3.7-fold and 4.3-fold vs. untreated control, respectively). Conversely, octanoic, myristic and linoleic acid displayed little to no effect. Cells were then treated with 2 fatty acids in combination, using only the 6 with the highest impact on PPAR activation, at the dose that maximizes PPAR activation. As hypothesized, the impact of 2 fatty acids in combination was greater than the sum of the activation of each individual fatty acid. The combinations of 3 fatty acids, each at a dose equal to one third of the optimal individual dose, resulted in a similar magnitude of effect on PPAR activation as compared with the combination of 2 fatty acids, while minimizing the total amount of fatty acids present in the medium. Our data indicates a greater nutrigenomic effect via PPAR of a mixture vs. single fatty acids, paving the way for the effective activation of PPAR isotypes in dairy cows through dietary means.

Key Words: long-chain fatty acids, peroxisome proliferator-activated receptors (PPAR), nutrigenomics

32 Effects of dietary organic acid and plant botanical supplementation on growth performance in Holstein calves challenged by heat stress. A. B. P. Fontoura*[1], V. Sáinz de la Maza-Escolà[1,2], B. N. Tate[1], J. T. Siegel Nieves[1], A. T. Richards[1], F. Wang[1,3], L. F. Wang[1,4], M. E. Van Amburgh[1], E. Grilli[2,5], and J. W. McFadden[1], [1]*Cornell University*, *Ithaca, NY*, [2]*University of Bologna*, *Bologna, Italy*, [3]*China Agricultural University*, *Beijing, China*, [4]*Henan Agricultural University*, *Zhengzhou, China*, [5]*VetAgro S.p.A*, *Reggio Emilia, Italy*.

Our objectives were to evaluate the effects of heat stress (HS) and dietary organic acid and plant botanical (OA/PB) supplementation on growth in calves. In a completely randomized design, 62 bull and heifer calves were assigned to 1 of 5 groups (n = 12–13/group): thermoneutral conditions (TN-Con), HS conditions (HS-Con), thermoneutral conditions pair-fed to HS-Con (TN-PF), HS with low-dose OA/PB (75 mg/kg of BW; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; AviPlus R; Vetagro, Italy; HS-Low), or HS with high-dose OA/PB (150 mg/kg of BW; AviPlus R; HS-High). Supplements were delivered as a twice daily bolus via the esophagus wk 1 through 13 of life; all calves received boluses equivalent for triglyceride. Post weaning, calves (62 ± 2 d; 91 ± 10.9 kg) remained in thermoneutral conditions (temperature-humidity index [THI]: 60 to 69) for a 7-d covariate period. Thereafter, calves remained in TN conditions or were moved to HS conditions (THI: 75 to 83) for 19 d. Clinical assessments and BW were recorded, and blood was sampled. Organs from HS-Con and TN-Con were harvested at trial completion. The mixed model included fixed effects of BW at birth, treatment, time, and their interaction. Rectal and skin temperatures, and respiration rates were greater in HS-Con, relative to TN-Con ($P < 0.01$). Dry matter intake (DMI) and average daily gain (ADG) were lower in HS-Con, relative to TN-Con ($P < 0.01$). Comparing HS-Con and PF-Con, ADG and gain:feed were similar. Plasma fatty acids were elevated in TN-PF versus all other groups ($P = 0.04$; not observed for HS-Con). Liver and small intestine weights were lower in HS-Con, relative to TN-Con ($P = 0.03$ and 0.15, respectively). DMI was greater with HS-Low, relative to HS-Con ($P < 0.01$). ADG for HS-Low and HS-High were not different from HS-Con or TN-Con (i.e., effect was intermediate). Compared with HS-Con, calves fed OA/PB tended have greater gain:feed ($P = 0.08$). We conclude that reductions in DMI account for losses in growth during HS and dietary OA/PB supplementation enhances HS resilience in calves.

Key Words: calf, heat stress, organic acid

33 Feeding rumen-protected lysine prepartum increased energy-corrected milk in Holstein cows during early lactation. L. K. Fehlberg*[1], A. R. Guadagnin[1], B. L. Thomas[1], Y. Sugimoto[2], I. Shinzato[2], and F. C. Cardoso[1], [1]*University of Illinois*, *Urbana, IL*, [2]*Ajinomoto Co. Inc*, *Tokyo, Japan*.

Balancing for AA in the diet can optimize milk yield and composition; however, there is little information on the requirement of AA, specifically Lys, during the transition period. This experiment was conducted to determine the effects of feeding rumen-protected Lys (RPL; AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) prepartum (0.54%DM of TMR), postpartum (0.395%DM of TMR), or both on performance. Seventy-five multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, expected calving date, and body condition score during the far-off dry period were assigned to 1 of 4 dietary treatments in a randomized, complete block design with a crossover of diet with RPL (L) or without (C). Treatments consisted of TMR with RPL prepartum and postpartum (LL), with RPL prepartum and without postpartum (LC), without RPL prepartum and with postpartum (CL), and without RPL prepartum and postpartum (CC). Cows were milked 2 × per d and milk samples were taken on 7 ± 1.3, 14 ± 1.4, and 28 ± 1.1 d relative to calving. Milk yield and dry matter intake (DMI) were obtained daily. Statistical analyses were performed using MIXED procedure of SAS. Cows in L had greater ($P = 0.03$) BW (823 ± 3kg for wk −2 and 785 ± 3kg for wk −1) during the −2 wk before calving compared with those in C (814 ± 3kg for wk −2 and 775 ± 3kg for wk −1). Postpartum BW (717 ± 6kg) was greater ($P = 0.05$) and DMI (18.12 ± 0.74kg) tended ($P = 0.08$) to be greater for cows in LL and LC compared with those that were in CL and CC (706.5 ± 6 and 16.84 ± 0.74 kg, respectively). Energy-corrected milk (48.7 ± 1.9 kg/d), 3.5% fat-corrected milk (50.1 ± 2.1 kg/d), milk fat (1.93 ± 0.09 kg/d), milk true protein (1.41 ± 0.05 kg/d), milk casein (0.64 ± 0.04 kg/d), and milk lactose yields (2.07 ± 0.08 kg/d) were greater ($P \leq 0.04$) for cows in LL and LC compared with those that were in CL and CC (44.2 ± 1.9, 45.2 ± 2.1, 1.71 ± 0.09, 1.30 ± 0.05, 0.54 ± 0.04, 1.88 ± 0.08 kg/d, respectively). In conclusion, cows that consumed RPL prepartum tended to increase DMI postpartum and increased energy-corrected milk and milk component yields.

Key Words: lysine, milk protein, transition period

34 Effects of rumen undegradable protein and amino acid sources and replacing forage or non-forage fiber in postpartum cows on production. A. W. Tebbe* and W. P. Weiss, *Ohio Agricultural Research and Development Center, The Ohio State University*, *Wooster, OH*.

Greater metabolizable protein (MP) supply from rumen undegradable protein (RUP) improves production in fresh cows. However, using one RUP source instead of blends may worsen AA imbalances as RUP (% of MP) increases. Replacing forage instead of non-forage fiber with RUP may also increase MP supply by increasing intake. Our objective was to determine whether high MP diets using one or a blend of RUP and AA sources and whether substituting forage NDF (fNDF) rather than non-forage NDF with RUP improves production in fresh cows. In a randomized block design, 40 primigravid and 40 multigravid Holsteins were blocked by calving date and fed a common diet (11.5% CP). After calving to 25 DIM, cows were fed 1 of 4 diets: 1) deficient MP (DMP, 17% CP, 24% fNDF), 2) adequate MP met with high inclusion of treated soybean meal (AMP, 20% CP, 24% fNDF), 3) adequate MP met with a blend of RUP and rumen protected AA (Blend, 20% CP, 24% fNDF), or 4) Blend but replacing fNDF rather than non-forage NDF with RUP (Blend-fNDF, 20% CP, 19% fNDF). Cows were fed a common diet (17% CP) from 26 to 92 DIM. Data were averaged by week and analyzed with models with fixed effects of diet, week (repeated), parity, their interactions and random effects of block nested within parity. During treatment, Blend vs. AMP tended to increase DMI (17.4 vs. 16.4 kg/d; $P = 0.098$) but not for Blend-fNDF (17.2 kg/d; $P = 0.79$). Blend and AMP had similar DMI from 26 to 50 DIM (19.2 vs. 20.1 kg/d; $P = 0.16$). For milk, fat and protein, AMP and Blend increased yields 2.1, 0.05 and 0.14 kg/d, respectively, vs. DMP ($P \leq 0.06$) but AA profile and fNDF interacted with parity. Blend vs. AMP had similar yields during treatment, but energy-corrected milk and fat yields increased ($P < 0.01$) 5.6 and 0.33 kg/d from 26 – 92 DIM in multiparous cows only. Blend-fNDF vs. Blend decreased ($P \leq 0.01$) milk and milk fat yields 2.8 and 0.32 kg/d in multiparous cows only. Lower milk fat yield ($P = 0.01$) carried over until 92 DIM (1.85 vs 1.65 kg/d). Blends of RUP and AA are better for fresh cows fed high MP, especially multiparous cows. Multiparous cows may also require more fNDF than primiparous cows.

35 Reproductive outcomes associated with delayed clinical cure of metritis in dairy cows.

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Approximately 20% of cows treated for metritis fail to resolve clinical signs shortly after antimicrobial therapy. Objectives were to evaluate reproductive outcomes, uterine health, and estrous cyclicity associated with delayed clinical cure of metritis. This prospective cohort study included data from 4 experiments performed between 2012 and 2018 in 6 Florida dairies. Metritis was characterized by presence of watery, fetid, reddish-brownish vaginal discharge (VD; VD = 5) within 21 DIM (d 0). Cows with metritis were treated with ampicillin or ceftiofur and paired with counterparts without metritis (NoMet; n = 2,906). On d 11, cows with metritis with VD <5 were classified as cured (MetC; n = 1,136) and those with VD = 5 were classified not cured (MetN; n = 279). Incidence of purulent vaginal discharge (PVD) was evaluated at 32 ± 7 DIM using the Metricheck device and estrous cyclicity was evaluated via 2 ultrasonographic exams 10 to 14 d apart, with the last exam between 50 and 67 ± 3 DIM. Binary variables were analyzed by logistic regression. Hazard of pregnancy and time to pregnancy were evaluated by Cox's proportional hazard regression models. Services per conception (SPC) was assessed by ANOVA. Incidence of PVD was greater ($P < 0.001$) for MetN compared with MetC and NoMet (93.2, 79.2, and 43.2%). Proportion of cyclic cows was smaller ($P < 0.02$) for MetN compared with MetC and NoMet (67.2, 79.9, and 91.8%). Pregnancy per AI (MetN = 28.2, MetC = 29.2, NoMet = 31.8%) and pregnancy loss (MetN = 13.1, MetC = 11.5, NoMet = 13.5%) after first AI did not differ ($P > 0.35$) among groups. SPC was greater ($P = 0.04$) for MetN compared with NoMet and intermediate for MetC (3.43, 2.98, 3.15). Hazard of pregnancy was smaller ($P < 0.001$) for MetN compared with MetC (AHR = 0.72; 95% CI = 0.49 to 0.94) or NoMet (AHR = 0.59; 95% CI = 0.49 to 0.70), and for MetC compared with NoMet (AHR = 0.82; 95% CI = 0.74 to 0.90). Mean days to pregnancy for MetN, MetC, and NoMet were 211, 183, and 170. Delayed clinical cure of metritis was associated with impaired subsequent uterine health, delayed resumption of ovulation postpartum, and decreased hazard of pregnancy.

Key Words: uterine disease, health, pregnancy

36 Variation in bovine colostrum fat content is related to specific lipid species.

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Colostrum is an essential source of nutrients, energy, and antibodies for neonates. Studies across multiple species associate long-term programming effects of colostrum on health, fertility and production capacity. However, the quality of colostrum varies extensively across cows and herds, including fat content, which is essential for calf temperature regulation and thermogenesis. Understanding what is potentially driving the variation of fat content in colostrum may enable the development of approaches to standardize quality to ensure nutrient needs of calves are met. The objective of this study was to determine if there is a relationship between the percent fat of colostrum and the amount of specific lipids in colostrum. Colostrum was collected from 16 multiparous cows within 2 h of calving and immediately frozen at −20°C until analysis. Colostrum fat percent was measured using the creamatocrit approach, 5.46 ± 2.20% (mean ± SD) and range from 0.92 to 8.36%. The Bligh and Dyer protocol was used to extract lipids from the colostrum and multiple-reaction monitoring (MRM) profiling was used to measure colostrum lipids on an Agilent 6410 QQQ mass spectrometer (Agilent Technologies). MRM-profiling was divided into 2 phases, a discovery and screening phase. For discovery phase a pooled colostrum sample was profiled, and lipids with intensities ≥1.3-fold in relation to the blank were selected for the screening phase. Individual colostrum samples were profiled for selected lipids. Data were uploaded into MetaboAnalyst 4.0 for statistical analysis following autoscaling for normalization. Correlation analysis, evaluating non-triglycerides, indicated a significant relationship between percent fat and multiple lipids in colostrum, including phosphatidylcholine (PC) 32:1 ($P = 0.004$, $r = 0.67$), PC 32:0 ($P = 0.02$, $r = 0.57$), PCo 40:0 ($P = 0.01$, $r = -0.62$), and PCo 42:4 ($P = 0.05$, $r = -0.50$), sphingomyelin (SM) d18:0/24:0 ($P = 0.02$, $r = 0.59$), and SM d18:1/24:0 ($P = 0.03$, $r = 0.55$). These findings suggest that specific colostrum lipids are related to percent fat, however further studies are required to determine regulatory mechanisms for colostrum fat synthesis.

Key Words: colostrum, fat, lipidome

37 Effect of hyperketonemia on circadian patterns of blood metabolites and milk predicted constituents in dairy cows.

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Estimates of milk and blood constituents by Fourier-transform mid-infrared (FTIR) analysis of milk offer a promising tool to monitor energy deficit in dairy cows. We sought to explore the (1) diurnal changes in plasma nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) and FTIR estimates of milk BHB and milk predicted blood NEFA (pbNEFA), (2) correlation between plasma BHB and NEFA, and (3) effect of hyperketonemia (HYK) on circadian patterns of plasma and milk metabolites. Multiparous Holstein cows (n = 28), between 3 and 9 DIM, were fit with jugular catheters and blood samples were collected every 2 h for 5 d. Cows were milked thrice daily (0600, 1400, 2200 h) and milk samples were collected at every milking for the same 5 d. Cows were fed daily at 0900 h and offered ad lib access to a TMR. Plasma NEFA and BHB was quantified by enzymatic analysis and milk BHB and pbNEFA was estimated by FTIR. Cows were retrospectively grouped as HYK positive (n = 13) if plasma BHB was ≥1.2 mmol/L for ≥3 study days or HYK negative (non-HYK; n = 15) if plasma BHB was ≥1.2 mmol/L for ≤2 study days. Explanatory models were used to analyze plasma and milk metabolites over time and differences in metabolites between HYK groups. Models analyzing metabolites over time included the random effect of cow and fixed effect of time; those analyzing differences between groups included the random effect of cow and fixed effects of HYK group, time, and HYK group × time.

The correlation between plasma NEFA and BHB was analyzed by calculating the area under the curve for total plasma NEFA and BHB. Plasma BHB and NEFA, milk BHB, and pbNEFA all changed throughout the day ($P < 0.001$). The amplitude of change in plasma BHB was greater within a day for the HYK cows than the non-HYK cows ($P = 0.009$). Plasma NEFA and BHB were positively correlated ($r = 0.81$), suggesting that accounting for diurnal variation increased the correlation of plasma metabolites. Our results support the use of FTIR estimates of milk constituents as a tool to monitor energy deficit and suggest that time relative to feeding should be considered when analyzing both plasma and milk metabolites.

Key Words: hyperketonemia, Fourier-transform mid-infrared (FTIR)

38 Isoprostanes reduce production of reactive oxygen species and apoptosis in a bovine model of oxidative stress.

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Oxidative stress is associated with several economically important diseases in dairy cattle and results in damage to tissue macromolecules. Isoprostanes (IsoP) are molecules generated from interactions between free radicals and membrane phospholipids, thus serving as excellent indicators of free radical-mediated lipid damage during times of oxidative stress. In dairy cattle, IsoP have been detected throughout the lactation cycle, during both health and disease. While IsoP are recognized as excellent biomarkers of oxidative stress, their physiological role remains largely unknown. The vascular endothelium is a primary target of lipid peroxidation during oxidative stress. Thus, this experiment aimed to determine the effect of the most extensively studied IsoP, 15-F2t-IsoP, on bovine endothelial cells during oxidative stress conditions. Bovine aortic endothelial cells (BAEC) were incubated in the presence of 10 nM 15-F2t-IsoP alone and in combination with known oxidizers 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and lipopolysaccharide (LPS). 15-F2t-IsoP decreased ROS production in BAEC incubated with AAPH for 12 h compared with cells incubated with AAPH alone. Additionally, 15-F2t-IsoP decreased apoptosis in BAEC incubated with LPS for 12 h when compared with cells incubated with LPS alone. The results of this study indicate that 15-F2t-IsoP may have a cytoprotective role during times of oxidative stress. Future studies should be directed toward investigating if IsoP alter other factors associated with vascular damage during oxidative stress, such as endothelial cell barrier integrity. This research benefits the industry by providing insight into how a well-known biomarker of oxidative stress in dairy cattle may contribute to the pathophysiology of economically important diseases.

Key Words: isoprostane, oxidative stress

Animal Health: Transition Cow 1

39 Effect of metritis on the pattern of behavioral, physiological, and performance parameters monitored by sensors in dairy cows. M. M. Pérez*, E. M. Cabrera, C. Rial, I. Foddanu, and J. O. Giordano, *Department of Animal Science, Cornell University*, *Ithaca, NY.*

Our objective was to compare the pattern of multiple behavioral, physiological, and performance parameters collected by sensors in Holstein cows diagnosed or not with metritis (MET). Cows underwent clinical examination daily from calving until 28 DIM. Sensor parameters monitored and collected were: physical activity (ACT), rumination (RUM) and eating (EAT) behavior, reticulo-rumen temperature (TEMP), and milk production (MILK) and milk components (fat and protein). Groups of cows were formed as follows: cows diagnosed with MET only (METO; n = 149), cows diagnosed with MET plus another disorder (MET+; n = 78) within 7 d before or after diagnosis of MET, and cows with no evident signs of clinical disease (NCD; n = 824). Data from d −7 to 7 after diagnosis of MET were analyzed by ANOVA with repeated measurements including group, time, and their interaction as fixed effects and controlling by lactation number (1, 2, 3+). The mean DIM at diagnosis of MET was considered study d 0 for cows in the NCD group. Cow within-group was included as random effect and was the subject of repeated measures. MILK was lower ($P < 0.01$) for cows in METO and MET+ from d −4 to 7. Cows in METO and MET+ had greater ($P < 0.01$) fat and fat-to-protein ratio than cows in NCD from d −7 to 7. For cows in METO and for MET+, ACT was lower ($P < 0.01$) than for cows in NCD from d −3 to −1 and from 1 to 7, respectively. EAT was reduced ($P < 0.01$) for cows in both METO and MET+ compared with NCD from d −6 to 7. Cows in MET+ had lesser ($P < 0.01$) RUM than cows in NCD from d −4 to 7 whereas cows in METO had lesser ($P < 0.01$) RUM than cows in NCD from d −4 to 4. Cows in METO had greater ($P < 0.01$) TEMP than cows in NCD from d −7 to 7, whereas cows in MET+ had greater ($P < 0.01$) TEMP than cows in NCD from d −1 to 7. We conclude that cows with metritis only and cows with metritis and another disorder observed within 7 d before or after diagnosis of metritis had specific patterns of behavioral, physiological and productivity parameters which might be used to predict the occurrence of metritis.

Key Words: metritis, sensor, dairy cow

40 Induced endometritis affects production in early lactation. A. Husnain*, U. Arshad, M. B. Poindexter, R. Zimpel, A. Vieira-Neto, Z. Ma, K. C. Jeong, W. W. Thatcher, C. Nelson, J. J. Bromfield, and J. E. P. Santos, *University of Florida*, *Gainesville, FL.*

Objectives were to investigate the impact of induced endometritis on performance in early lactation. The hypothesis was that induced endometritis induces localized and systemic inflammatory responses that depress production. Postpartum Holstein cows without any clinical disease in the first 30 DIM and less than 10% polymorphonuclear cells (PMN) in endometrial cytology had the estrous cycle synchronized starting at 29 DIM. Cows were blocked by parity and genomic breeding value for fertility and, within block, assigned randomly to remain as untreated controls (CON; n = 19) or to receive an intrauterine infusion of $5x10^8$ colony-forming units of *Escherichia coli* and 5×10^8 colony-forming units of *Trueperella pyogenes* on d 6 of an estrous cycle at 44 DIM (INF; n = 19). Endometrial cytology was taken on d 2 and 7 after treatment to evaluate PMN and mRNA expression of inflammatory genes. Rectal temperature, milk yield and components were evaluated from d −7 to 7 relative to treatment. Data were analyzed by mixed models with the MIXED and GLIMMIX procedures of SAS. Treatment increased ($P < 0.01$) the proportion of PMN in the endometrial cytology on d 2 (CON = 3.9 ± 1.1 vs. INF = 39.9 ± 6.6%) and 7 (CON = 8.5 ± 2.2 vs. INF = 19.0 ± 4.3%) after infusion. Using a cut-off of 10% PMN as subclinical endometritis (SCE), 28.7 ± 9.8 of CON and 91.9 ± 5.3 of INF developed SCE ($P < 0.001$). Rectal temperature did not differ ($P = 0.52$) between CON and INF and averaged 38.9 and 38.9 ± 0.1°C, respectively. Yield of milk in the 7 d following treatment tended ($P = 0.09$) to be greater in CON than INF (CON = 44.0 vs. INF = 40.9 ± 1.8 kg/d). Treatment did not affect the yields of ECM (CON = 42.8 vs. INF = 41.9 ± 1.9 kg/d; $P = 0.65$), fat (CON = 1.45 vs. INF = 1.52 ± 0.07 kg/d; $P = 0.45$), or protein (CON = 1.22 vs. INF = 1.14 ± 0.07 kg/d; $P = 0.20$), but lactose yield was greater ($P = 0.02$) in CON than INF (CON = 2.37 vs. INF = 2.09 ± 0.12 kg/d). The somatic cell score (SCS) did not differ ($P = 0.95$) between treatments (CON = 2.7 vs. INF = 2.7 ± 0.3). Induction of endometritis induced an influx of PMN, increased incidence of SCE and affected yields of milk and lactose in early lactation cows.

Key Words: dairy cow, endometritis, production

41 Assessment of the accuracy and test characteristics of a multivariable metabolic index to predict hyperketonemia in early postpartum Holstein dairy cows. Z. Rodriguez[1], L. Caixeta*[1], P. Ferro[1], N. Moraes[1], M. Endres[1], K. Nakagawa[2], A. Imaizumi[2], I. Shinzato[2], and T. Fujieda[2], [1]*University of Minnesota*, *Saint Paul, MN*, [2]*Ajinomoto Co. Inc*, *Kawasaki, Japan*.

Hyperketonemia (HYK) is a prevalent metabolic disorder associated with suboptimal health and performance in dairy cattle. Thus, early detection and prevention of HYK are important to improve animal welfare and decrease economic losses. A multivariable metabolic index (MI) using pre-partum concentration of different amino acids and blood metabolites was developed to predict the occurrence of HYK in Holstein dairy cows. Our objective was to assess the accuracy of this MI and the variability of test characteristics at different MI thresholds. Blood samples were collected from Holstein cows in a commercial dairy in Minnesota at 21 ± 3 d before expected calving date (3 weeks pre-partum) for the measurement of blood metabolites and calculation of the MI. After calving, blood β-hydroxybutyrate (BHB) was measured at 3 ± 1 and 7 ± 1 DIM for diagnosis of HYK. Hyperketonemia was defined as BHB >1.2 mmol/L. Cows were enrolled between February and September 2019. Statistical analyses were performed using R software (version 3.4.4). The area under for the receiver operating characteristic curve was calculated as a measure of predictive accuracy. Sensitivity (Se), specificity (Sp), positive predictive values (PPV) and negative predictive values (NPV) at 6 different predictive thresholds were calculated as test characteristics. The prevalence of HYK was 19.0% (95%CI: 15.9 – 22.5). The diagnostic accuracy of the MI to predict HYK was 0.79 (95%CI: 0.73 – 0.85). The best Se and Sp based on Youden's index were 75% and 76%, respectively based on d 7 (Table 1). In conclusion, the MI was able to predict HYK with high accuracy and acceptable Se and Sp up to 3 weeks before the disease onset on the postpartum period.

Table 1 (Abstr. 41). Test characteristics[1] at different metabolic index (MI) thresholds

MI Threshold	Se (%)	Sp (%)	PPV (%)	NPV (%)
−0.7	45	90	40	92
−1.1*	75	76	31	95
−1.2	79	70	28	96
−1.5	88	50	21	97
−1.8	92	30	16	96
−2.3	96	10	14	95

[1] Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value.

*Represents the MI value with the highest Youden's index.

Key Words: hyperketonemia, prediction, transition period

42 The association of prepartum urine pH, and plasma total calcium at calving in Holstein dairy cows. P. Melendez*[1], J. Bartolome[2], C. Roeschmann[3], B. Soto[2], A. Arevalos[4], and J. Moller[4], [1]*University of Georgia*, *Tifton, GA*, [2]*National University of La Pampa*, *Gral. Pico, Argentina*, [3]*University of Chile*, *Santiago, Chile*, [4]*Fundo Los Laureles*, *Chahuilco, Chile*.

Hypocalcemia is a common metabolic disorder affecting dairy cows around parturition. A successful strategy to prevent clinical hypocalcemia is the use of anionic diets to induce a mild metabolic acidosis, increasing the responsiveness of receptors to PTH. The assessment of urine pH is a quick and inexpensive tool to monitor the degree of metabolic acidosis imposed by the anionic strategy. What urine pH is the ideal for the prevention of milk fever has been very controversial. The aim was to assess urine pH on prepartum Holstein cows fed anionic diets and determine its association with plasma total Ca (tCa), tMg, P, BHB at parturition. The study was conducted on a grazing dairy in Chile. At 30 d before expected parturition, cows were moved to a prepartum lot receiving 80% of their DM as a mixed ration and 20% from pasture (DCAD −109 mEq/kg DM). During Fall 2019, 345 prepartum cows were eligible for urine collection. Weekly, about 20% of the group were randomly sampled for urine pH using a portable electronic pH meter. The inclusion criteria of each sampled cow was that they had to stay at least one week in the prepartum group consuming the anionic diet. Within 6 h from calving a blood sample was obtained to collect plasma. At the end, 60 cows were tested for urine pH and blood metabolite concentrations at calving. The concentration of plasma tCa, P, tMg and BHB at d 1 pp were assessed. Metabolites were analyzed by ANOVA for polynomial regression (PROC GLM, SAS 9.4). Ten cows (16.6%) had a urine pH <6.0, 35 cows (58.3%) had a tCa concentration <2.15 mmol/L. There was a quadratic effect of urine pH on tCa. The concentration of plasma tCa was higher when the prepartum urine pH was between 6.0 and 7.5 (2.24–2.3 mmol/L), while decreased with pH <6.0 and > pH 7.5 (<2.22 mmol/L). No variables were associated with tMg at calving. There was a trend ($P = 0.11$) for a quadratic effect of urine pH on the concentration of BHB at parturition. BHB was higher when urine pH was <6.0 and >7.5. In conclusion, cows with prepartum urine pH <6.0 and >7.5 had a lower concentration of plasma tCa, and tended to have a higher concentration of BHB. These results indicate that anionic salts should be fed with caution, avoiding underfeeding and overfeeding. The target urine pH should be between 6.0 and 7.0.

Key Words: hypocalcemia, urine pH, anionic diets

43 Association of calcium and energy balance metabolites in the first 3 days after parturition with disease and production outcomes in multiparous Jersey cows. P. R. Menta*[1], L. Fernandes[1], D. Poit[1], M. Celestino[1], V. S. Machado[1], M. A. Ballou[1], and R. C. Neves[1,2], [1]*Department of Veterinary Sciences, College of Agricultural Sciences and Natural Resources, Texas Tech University*, *Lubbock, TX*, [2]*Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University*, *West Lafayette, IN*.

Our objectives were to understand the associations of total Ca concentration (tCa) measured at 1 through 3 DIM, and free fatty acids (FFA), BHB and glucose (GLU) measured at DIM 3 with (1) the risk of multiparous Jersey cows being diagnosed with early-lactation diseases and culling using multivariable Poisson regression; (2) milk production in the first 9 wk of lactation using generalized linear mixed models; and, (3) the risk of pregnancy in the first 150 DIM using Cox Proportional hazards modeling. A total of 380 cows were enrolled in a cohort study from one herd in West TX. Total Ca measured at DIM 1 through 3 was not associated with the risk of metritis. Cows with increased FFA and BHB had a higher risk to be diagnosed with metritis ($P = 0.05$) and clinical mastitis ($P = 0.04$), respectively. Increased concentrations of GLU ($P = 0.007$), FFA ($P = 0.04$), and tCa ($P = 0.004$) at 3 DIM were associated with the risk of culling. Reduced tCa at both DIM 1 (≤1.84 mmol/L; $P < 0.0001$; +3.85 kg per d) and 2 (≤1.88 mmol/L; $P = 0.03$; +1.84 kg per d) were associated with increased milk production across the first 9 wk of lactation compared with herdmates with increased tCa. Total Ca was not associated with milk production at DIM 3, with FFA (≥0.55 mmol/L; $P = 0.02$) and GLU (≤2.95 mmol/L; $P = 0.002$) being associated with increased milk production. None of the metabolites measured were associated with the risk of pregnancy in the first 150 DIM. Our results demonstrate that reduced tCa concentrations in the first 2 DIM and reduced GLU at 3 DIM are associated with increased milk production. Increased concentrations of FFA were associated with higher milk production but with increased risk of metritis.

Key Words: calcium, energy balance, milk production

44 Effects of carprofen on inflammation biomarkers and health of transition Holstein cows. S. T. Quanz*[1], A. R. Rodríguez[2], H. A. Bustamante[2], L. K. Mamedova[1,3], and B. J. Bradford[1,3], [1]*Kansas State University*, *Manhattan, KS*, [2]*Universidad Austral de Chile*, *Valdivia, Los Rios Region, Chile*, [3]*Michigan State University*, *East Lansing, MI*.

The objective of this study was to analyze the effect of carprofen on transition cow inflammation biomarkers and health. Late-gestation multiparous cows (n = 62) were enrolled at the beginning of the close-up period and randomly assigned to treatments at parturition. Cows received either no treatment (control) or subcutaneous carprofen (1.4 mg/kg BW) 24 – 48 h postpartum. Cows treated with antibiotics or anti-inflammatories during the close-up period were removed from the study (6 treatment, 2 control). Health was observed daily for 21 d postpartum. For the first 120 d of lactation, cows were confined and fed a TMR, and then moved to a rotational grazing system and supplemented. Blood plasma and serum were collected from the coccygeal vein 21 d before expected calving and once weekly for 3 wk postpartum. Serum haptoglobin was determined by enzymatic activity, plasma fibrinogen and total protein concentrations by refractometry, and plasma maresin-1 and adiponectin concentrations by ELISA. Treatment, disease, and time were modeled as fixed effects with parity as a random effect; significance was declared at $P < 0.05$. Treatment cows had fewer instances of clinical metritis (36% vs. 66%, $P \leq 0.05$), but there was no effect on puerperal metritis, displaced abomasum, pneumonia, clinical mastitis, or lameness incidence ($P > 0.10$). Carprofen had no effect on adiponectin or fibrinogen concentrations ($P > 0.10$) but increased total plasma protein concentration ($P \leq 0.05$) and tended to increase maresin-1 ($P = 0.09$), a resolving oxylipid. Carprofen tended to decrease plasma haptoglobin ($P = 0.07$). Cows with clinical metritis had greater concentrations of fibrinogen and haptoglobin ($P \leq 0.05$), but there were no disease × treatment interactions ($P > 0.10$). Carprofen tending to increase adiponectin concentration only among cows with clinical metritis ($P = 0.06$; disease × treatment interaction $P < 0.05$). Overall, carprofen decreased the incidence of clinical metritis while also altering biomarkers in a manner suggesting decreased systemic inflammation, consistent with a more successful navigation of the transition period.

Key Words: metritis, maresin-1, adiponectin

Growth and Development

45 The effect of fecal microbiota transplants in pre-weaned dairy calves. G. S. Slanzon*, L. M. Parrish, S. C. Trombetta, W. M. Sischo, and C. S. McConnel, *Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University*, *Pullman, WA*.

The GI tract of dairy calves contains a complex community of microorganisms that can be disrupted by GI disease. Fecal microbiota transplants (FMT) may be an alternative for restoring the GI microflora. The aim of this randomized controlled trial was to evaluate the effects of FMT on the fecal microbiome in pre-weaned dairy calves on a calf ranch in the western US. FMT product was made from *Salmonella* negative, concentrated fecal matter obtained from healthy calves (5–24 d of age) several weeks before this study and described elsewhere. Calves (n = 151) were randomly assigned to FMT treatment groups, clinically assessed twice daily from 1 to 21 d of age, and enrolled in the trial from 4 to 12 d of age. At enrollment, calves with fecal scores ≤ 2 and no clinical illness were classified as healthy. Those with fecal scores of 3 (loose) or 4 (watery) were diagnosed with diarrhea and classified as bright-sick (BS) or depressed-sick (DS) according to their behavior. Fecal samples were collected 10 d after enrollment (13 to 21 d of age) from calves that did (n = 82) or did not (n = 69) receive FMT (35g PO SID for 3 d). The V3-V4 region of the 16S rRNA gene was sequenced from fecal samples and analyzed using the DADA2 pipeline. An ASV table was used to compare taxonomic profiles and differences were identified by LEfSe ($P < 0.05$; LDA score > 2). The genus *Lactobacillus* was abundant in healthy calves that remained healthy post-FMT. Healthy calves that remained healthy without FMT had an abundance of the family *Actinomycetaceae*. Healthy calves that progressed to BS without FMT had an abundance of the family *Lachnospiraceae*. Healthy calves that progressed to BS post-FMT had an abundance of the family *Lactobacillaceae*. A chi-squared test of independence examined the relationship between FMT administration and changes in clinical outcomes. The proportion of initially healthy calves that progressed to BS, DS or died did not differ by FMT administration ($P = 0.14$). However, BS and DS calves without FMT treatment were more likely to recover to a healthy state (p-value = 0.02), necessitating a reconsideration of the characteristics and utility of FMT in dairy calves.

Key Words: fecal microbiota transplant, calf

46 Seaweeds supplementation affects the short-chain fatty acids profile in digesta from milk-fed Holstein calves. M. B. Samarasinghe*, M. Vestergaard, J. Sehested, T. Larsen, and L. E. Hernández-Castellano, *Department of Animal Science, Aarhus University, Foulum*, *Tjele, Denmark*.

This study aimed to determine the effects of supplementing 3 different seaweeds on ADG, organs' weight, pH and short chain fatty acids (SCFA) profile in digesta from milk-fed calves. Forty Holstein calves in 10 blocks (4 animals/block) with birth BW 41 ± 4 kg and plasma BRIX% ≥ 8.7% at d 2 after birth were used. From d 2 until d 49, control calves (CTRL, n = 10) received cow milk without seaweeds twice per day (8L/d). Experimental calves received cow milk supplemented with either *Ulva lactuca* (SW1, n = 10), *Ascophyllum nodosum* (SW2, n = 10), or *Laminaria saccharina* (SW3, n = 10). Dried and ground seaweeds were offered in a concentration of 25 g/4 L of milk twice daily. Calves were slaughtered either on wk 5 (n = 20) or wk 7 (n = 20) of life. After slaughtering, the small intestine was divided into 3 equal sections (i.e., SI1, SI2 and SI3). Digesta from SI2 and mid-colon (Mcol) was used to determine the SCFA profile. In addition, organs' weight and digesta pH were recorded. The statistical model included a one-way ANOVA with treatment as fixed effect and block as covariate. No differences in ADG, proportional organs' weight and digesta pH were detected ($P > 0.05$). Total SCFA concentration in Mcol digesta tended to be lower in SW1 (57.6 mmol/L) and SW3 calves (55.6 mmol/L) than in CTRL calves (78.8 mmol/L) on wk 5 ($P = 0.06$). However, no differences were detected in total SCFA concentration in SI2 digesta from any of the groups on wk 5. Acetic acid percentage in Mcol digesta was higher in SW2 calves than in CTRL calves on both wk 5 (71.4 vs. 65.7%, respectively) and wk 7 (74.2 vs. 63.3%, respectively) ($P < 0.05$). Additionally, propionic acid percentage in Mcol digesta was lower in SW2 calves (13.8%) compared with CTRL calves (21.2%) on wk 7 ($P < 0.05$). Furthermore, butyric acid percentage in Mcol digesta was lower in SW1 (5.62%) and SW2 calves (6.46%) than in CTRL calves (8.68%) on wk 7 ($P < 0.05$). In conclusion, compared with control, seaweeds supplementation increased the acetic acid percentage and reduced the propionic and butyric acid percentage in mid-colon digesta from milk-fed Holstein calves on wk 7 of life.

Key Words: seaweed, short-chain fatty acids, calf

47 Supplementation of seaweeds improves the immune status of milk-fed Holstein calves. M. B. Samarasinghe*, M. Vestergaard, J. Sehested, T. Larsen, and L. E. Hernández-Castellano, *Department of Animal Science, Aarhus University, Foulum*, *Tjele, Denmark*.

Seaweeds are used as functional feed ingredients as they contain a variety of complex polysaccharides, vitamins, chelated minerals and antioxidants, which are known to promote immune status, hence health and growth of humans and livestock. This experiment aimed to study the systemic immune status of calves fed milk supplemented with either *Ulva lactuca*, *Ascophyllum nodosum,* or *Laminaria saccharina.* Forty Holstein calves in 10 blocks of 4 with birth BW 41 ± 4 kg and plasma BRIX% ≥ 8.7% 2 d after birth were used. Calves were fed 4 L of cow milk twice a day (8 L/day). From d 2 until d 28, calves in the control group (CTRL, n = 10) received milk without seaweed. Experimental calves received milk supplemented with either *Ulva lactuca* (SW1, n = 10), *Ascophyllum nodosum* (SW2, n = 10) or *Laminaria saccharina* (SW3, n = 10). Dried and ground seaweeds were offered in a concentration of 25 g/4 L of milk. Blood samples were collected from the jugular vein on d 2, 4, 7, 14, 21, and 28 after birth. Plasma concentrations of immunoglobulins, serum amyloid A (SAA) and tumor necrosis factor α (TNF-α) were analyzed by ELISA, fibrinogen (Fib) by immune reaction and turbidimetry, and total protein, albumin and haptoglobin by spectrophotometric methods. The statistical model included the effect of treatment, sampling day, and their interaction, block and calf. Autoregressive covariance structure was applied for repeated measurements. No differences in ADG were detected between the groups (0.9 ± 0.03 kg/day) during this study. However, plasma concentrations of Fib ($P = 0.05$) and SAA ($P = 0.04$) were significantly different between treatment groups and there was a tendency for TNF-α ($P = 0.07$). At d 14, Fib was significantly higher in calves fed with SW1 (5.7 ± 0.5 g/L) and SW2 (5.4 ± 0.5 g/L) compared with the CTRL group (3.9 ± 0.5 g/L). In addition, plasma SAA was significantly higher in SW2 (181 ± 31 mg/L) and SW3 (214 ± 31 mg/L) calves than in CTRL calves (103 ± 31 mg/L) at d 14. These results indicate that milk supplementation with seaweeds improves the systemic immune status in milk-fed calves.

Key Words: seaweed, immunity, calf

48 The effect of tributyrin supplementation to milk replacer on growth performance, diarrhea frequency, and blood biochemical parameters of dairy calves. S. Liu*, J. Zhou, J. Wu, J. Ma, and Z. Cao, *State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University*, *Beijing, China*.

The aim of this study was to evaluate the effect of rumen unprotected tributyrin supplementation to milk replacer on performance, diarrhea frequency and blood concentrations of metabolite of dairy calves. Forty-eight newborn Holstein female calves (39.6 ± 2.7 kg of BW; mean ± SD) were randomly allocated to 3 treatments to assess the effects of supplementing the milk replacer at 2.8 g/kg (TB_8) or 5.6 g/kg (TB_{16}) of powder with tributyrin compared with no supplementation (CON). Calves were weaned on d 56 and were raised until the end of this study (d 77). Starter intake, fecal consistency scores, BW and blood were collected. Data were analyzed separately by stage: pre-weaning (d 1 - d 56) and post-weaning (d 57 – d77). All repeated, continuous data were analyzed using the MIXED procedure of SAS. The results showed that during the pre-weaning period, calves in TB_8 and TB_{16} had higher dry matter intake compared with CON, and the average daily gain of TB_{16} was higher than that of CON. After weaning, calves in TB_{16} had higher dry matter intake compared with CON, and all treatments had similar average daily gain. Tributyrin addition reduced the diarrhea frequency throughout the study. The results related to blood showed that no differences were found among treatments on concentrations of growth hormones, insulin and total protein throughout the study. The concentration of β-hydroxybutyrate in TB_{16} was higher than that of CON and TB_8 in wk 11, and no differences were found in other time points. The concentrations of plasma endothelin and serum amyloid A in CON increased after weaning, and plasma endothelin concentration was higher than TB_{16} in wk 9. In conclusion, rumen protected tributyrin supplementation to milk replacer could improve growth performance and reduce diarrhea frequency of calves. Supplementing the milk replacer at 5.6 g/kg of powder performed better.

Key Words: calf, tributyrin

49 Effects of feeding *Saccharomyces cerevisiae* fermentation products on the health and growth performance of Holstein dairy calves through 4 months of age.

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Our objective was to evaluate the effect of *Saccharomyces cerevisiae* fermentation products (SCFP) on growth and health of calves in the first 4 mo of life. Holstein bull calves were blocked by BW and serum total protein and assigned to 1 of 2 treatments; CON (24% CP:17% fat milk replacer (MR), 18% CP starter, 18% CP grower, and 16% CP grower with no SCFP added) or SCFP (24% CP:17% fat MR with 1 g/d of SmartCare (Diamond V), 18% CP starter with 0.8% NutriTek (Diamond V), 18% CP grower with 0.44% NutriTek, and 16% CP grower with 0.275% NutriTek). Calves were offered 2.84 L (12.5% solids) of MR twice daily (0630 and 1630 h) through d 51, from d 52–56 calves were fed MR once daily (0630 h), and weaned on d 56. From d 1–56, calves received ad libitum access to a texturized calf starter and water. On d 57, calves were switched to an 18% CP grower and on d 71, calves were switched to a 16% CP grower. Individual calf BW, BCS, hip height (HH), and hip width (HW) were measured biweekly from d 0–112. Intake was recorded daily and feed efficiency (FE) and ADG were calculated. Daily fecal and respiratory scores were recorded for each calf through d 56 and all medical treatments were recorded for the duration of the study and grouped based on illness. Data were analyzed as a completely randomized block design in SAS v.9.4 with repeated measures when applicable. No treatment effect was observed for BW, BCS, HH, or HW at d 0, d 56, or d 112 (all $P \geq 0.11$). A treatment tendency was observed for post-weaning ADG ($P = 0.07$) with SCFP calves having higher ADG compared with CON calves. SCFP calves had improved FE compared with CON post-weaning ($P = 0.02$). A treatment effect was seen for respiratory illness ($P = 0.001$), with SCFP calves being treated less frequently than CON (0.95 vs. 0.31 treatments/calf). This study suggests that feeding *Saccharomyces cerevisiae* fermentation products to calves could improve ADG and FE post-weaning as well as reduce the incidence of respiratory disease through 4 mo of life.

Key Words: calf health, *Saccharomyces cerevisiae* fermentation product, growth

50 Evaluation of Lifeline Protect at arrival and NutraPro formulated into a traditional milk program under California feeding and housing conditions during the winter season on calf growth, morbidity, and mortality.

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Several environmental factors affect calf's health, including nutrition. Functional plasma proteins (FPP) fed to neonatal calves have been reported to improve performance and reduce fecal scores, medical treatments and mortality. However, research evaluating Lifeline Protect (colostrum supplement) (LP) and NutraPro (spray-dried plasma, NP) in a traditional California milk and housing program is limited. The objective was to evaluate a FPP program consisting of LP upon arrival and NP added to the milk feeding program for dairy calves during cold stress on performance, morbidity, and mortality. Milk fed to calves was a mixture of whole milk and milk replacer fortified to 14% solids. A step-down program was used so that d-56 calves were weaned. Holstein bull calves (n = 54) were sourced from 8 different dairies and transported to Fresno State University campus on January 2019. Calves were randomized to 1 of 2 feeding treatment groups based on dairy origin, serum total protein level and BW, and then housed in wooden, 3-hole calf hutches. Treatments groups consisted of a control milk program (CMP) using whole milk with added milk replacer to balance solids to 14% and the FPP program consisting of LP (50 g globulin/calf) upon arrival and thereafter using whole milk with 5% NP plus milk replacer to 14% solids. Weekly BW was recorded. Bottles were weighed before and after each feeding to determine milk intake. Daily grain consumption was recorded. Twice daily health and fecal scores were taken to evaluate morbidity. In the first 14 d, calves fed FP were heavier ($P < 0.05$: 44.8 vs. 43.5 kg) compared with CMP calves. By 56 d, FP calves were 74.5 kg vs 73.9 kg for calves fed CMP. Over the 8-week period, attitude score, average fecal score and number of medications per calf (7.3 vs. 12) was reduced ($P < 0.05$) for calves fed FPP compared with CMP. Survival was numerically increased for calves fed FPP (92%) vs CMP (80.8%). Calves fed FPP performed better and had less medication treatments, morbidity, and mortality compared with calves on CMP.

Key Words: plasma

51 Effects of transition milk on intestinal development of neonatal calves.

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Transition milk (TM, milk from the 2nd through 4th milkings after calving) feeding in the first week of life increases BW gain throughout the preweaning period. The objective of this study was to determine if TM enhances intestinal development compared with milk replacer (MR). We tested this idea on 23 newborn Holstein bull calves, born 6 different weekends within 12 h of each other per week. Calves were fed 2.8 L of colostrum within 15 min of birth randomly assigned to MR or TM treatments within block and fed treatments 3 times per day. TM was collected, pooled by milking number, and fed at 1.89 L (255 g DM) per feeding as follows: milking 2 at feedings 2 to 5, milking 3 at feedings 6 to 8, and milking 4 at feedings 9 to 12. In this study, TM was not pasteurized and had an average composition of 30% fat and 39% protein on a DM basis and 20 g IgG/L. Calves fed MR received 275 g DM MR (21% fat and 27% protein) at all 12 feedings. Both treatments had average refusals of 10%. At 0730 h on d 5, calves were injected IV with 5 mg of bromodeoxyuridine per kg BW and euthanized 130 min later. Sections of the duodenum, proximal jejunum, mid jejunum, and ileum were excised to evaluate morphology. Compared with MR, TM nearly doubled villus length, villus width, villus to crypt ratio, and mucosal length in all sections ($P < 0.01$ for all). Compared with MR, TM increased submucosal thickness 70% in the proximal and mid jejunum ($P < 0.001$) and tended to increase submucosal thickness in duodenum and ileum ($P < 0.1$). Treatment did not alter crypt depth. In all sections, labeling with bromodeoxyuridine was

increased 50% ($P < 0.01$ for all) by TM compared with MR in the cells along the epithelium of the crypts and within the villi, indicating that TM increased cell proliferation compared with MR. We conclude that feeding TM enhances development of the small intestine in the first few days of life compared with MR. This increased development might explain why calves fed TM compared with MR in the first 4 d of life grow faster.

Key Words: calf, transition milk, intestinal development

Lactation Biology

52 Disruption of circadian clocks in the prepartum dry period negatively affect mammary development. K. Teeple*[1], A. Suarez-Trujillo[1], C. McCabe[1], J. Townsend[2], S. Donkin[1], K. Plaut[1], J. Boerman[1], and T. Casey[1], [1]*Department of Animal Sciences, Purdue University*, *West Lafayette, IN*, [2]*College of Veterinary Medicine, Purdue University*, *West Lafayette, IN.*

Circadian clocks generate 24-h rhythms of physiology and behavior to coordinate internal metabolism and synchronize it to the environment. Studies of rodents indicate that disruption of circadian clocks negatively impacts mammary development. Our objective was to disrupt circadian clocks of cattle by exposing them to light (L)-dark (D) cycle phase shifts during the prepartum nonlactating dry period, and determine the effect on mammary development. Multiparous cattle were dried off at 60 d before expected calving (BEC) and assigned to control (n = 6) or phase shifted (PS; n = 6) treatments. Control were exposed to 16 h of L: 8 h D. PS group light exposure shifted forward 6 h every 3 d. Both groups began treatments 35 d BEC and stayed on respective treatments until calving. Following parturition all cows were exposed to 16:8 h L:D cycle. Mammary biopsies were taken at 21 d BEC and 21 d postpartum (PP). Biopsies were fixed in 10% formalin, embedded in paraffin, and sectioned for hematoxylin and eosin staining and immunostained for proliferating cell marker KI67. Images (n = 5/biopsy/cow) of tissue were captured at 40X to measure alveolar, lumen, and intralobular stromal area using ImagePro Plus 5.1. To determine proliferation index of mammary epithelial cells (MEC) and stromal cells, the total number of KI67 stained and unstained cells were counted (5 images/biopsy/cow) at 200X. The lumen to epithelial ratio was calculated by dividing the luminal area by the alveolar area. Milk yield was measured daily through 60 d PP. SAS (V 9.4) PROC MIXED was used for statistical analysis. Proliferation index of MEC at 21 BEC was lower ($P < 0.05$) in PS (5.2% ± 0.8) vs control (12.4% ± 0.8) cows; there was no difference in proliferation rates at 21 PP (C = 0.77% ± 0.75; PS = 0.78% ± 0.70). Alveolar area was different between time points ($P = 0.002$). Treatment and time point affected lumen to epithelial ratio ($P < 0.05$). Milk production was lower ($P < 0.05$) in PS (40.3kg ± 0.803) vs control (42.5kg ± 0.803). Findings support that phase shifting the L:D cycle alters mammary development during the prepartum period, and indicate that disruption of clocks may affect milk production.

Key Words: circadian, mammary development

53 Light-dark phase shift circadian disruption does not affect cortisol and progesterone concentrations in periparturient dairy cows. A. Suarez-Trujillo*[1], J. Franco[2], T. Steckler[1], J. Boerman[1], K. Plaut[1], and T. M. Casey[1], [1]*Department of Animal Sciences, Purdue University*, *West Lafayette, IN*, [2]*Metabolite Profiling Facility, Bindley Bioscience Center, Purdue University*, *West Lafayette. IN.*

Circadian-metabolic-reproductive systems are integrated and reciprocally regulated. Circadian clocks set the daily rhythms of multiple hormones that influence metabolism and reproduction. Currently, there is little understanding regarding how reproductive state effects circadian clock function, and how circadian clocks regulate metabolism during pregnancy and lactation. A previous study showed that exposure to chronic light-dark phase shifts (PS) during the dry period in dairy cows resulted in loss of the centrally generated circadian rhythms of melatonin and core body temperature, as well as decreased blood glucose and increased overall melatonin levels. These changes were related to increased milk production postpartum. The objective of this study was to measure cortisol (CORT) and progesterone (P4) in the transition period dairy cows exposed to PS to determine how altering central clock function effects these metabolic and reproductive hormones. Beginning 5 wk before expected calving (BEC) multiparous cows were assigned to control (n = 16) and exposed to 16h light: 8h dark or phase-shifted (n = 16) by 6h every 3d until parturition. All cows were exposed to control lighting after calving. Blood samples were taken at 0600 on d 35 BEC, 21 BEC, 2 BEC, and d 0, 2, 9, 15 and 22 postpartum. A subset of cows (n = 6/group) were sampled every 4 h over 48 h at 3 time points: d 23 BEC, 9 BEC, and 5 DIM, and data was analyzed for fit to 24 h rhythm in R cosinor analysis. CORT and P4 were measured using LC MS/MS. Two-way ANOVA analysis of the concentration of CORT and P4 found no effect of treatment (CORT $P = 0.48$; P4 $P = 0.52$). Cosine analysis of CORT and P4 concentration determined that neither treatment showed circadian rhythms in the 3 studied periods. Results suggest that CORT or P4 levels cannot account for treatment differences in milk yield and glucose. Lack of circadian rhythms in of hormone levels suggest that in periparturient cows 24 h profiles of CORT and P4 levels reflect reproductive state, and this milieu may mask circadian rhythms.

Key Words: circadian rhythms, cortisol, progesterone

54 Mammary gland transcriptome profiling reveals an abundance of transcripts for cell maintenance and protein turnover. L. Beckett*, J. Thimmapuram, S. Xie, T. Casey, and S. S. Donkin, *Purdue University*, *West Lafayette, IN*.

Identifying gene networks that support milk synthesis is critical to understanding mechanisms that control efficient milk production and response to nutrient supply. The objective of this study was to analyze the coding mammary transcriptome in early lactation cows across mag-

Table 1 (Abstr. 54). Magnitude of read count categories for functional annotation analysis

Magnitude of normalized read counts	No. of Genes	% Reads	Ontological category
≥50k	13	67	Milk protein
<50k to ≥5k	147	6.9	Translation
<5k to ≥1k	813	6.6	Glycolysis
<1k to ≥500	1,225	3.7	Proteasome
<500 to ≥200	2,850	4.0	Ubiquitin
<200 to ≥100	2,314	1.5	Mitochondria
<100 to ≥50	1,858	0.6	DNA repair
<50 to ≥10	2,761	0.3	Immune response
<10 to ≥5	749	0.023	Complement cascades
<5	3,803	9.4	—
Total	16,533	100	

nitudes of normalized RNA-seq read counts as a relative indicator of their importance in supporting milk synthesis. Forty-five mL of milk was collected from multiparous cows (n = 6; DIM 68 ± 3 d) following 2 treatment periods of a post-ruminal lysine infusion of 0 and 63 g/d for 10 d, which was part of a larger study. Total RNA was isolated (n = 12 samples), libraries prepared, and paired end reads were sequenced on an Illumina HiSeq2500 platform. Of the total reads (236M ± 39M), 79% mapped to the ENSEMBL bovine genome. EBseq analysis found no significant effect of lysine infusion on the transcriptome, therefore, reads were averaged across both treatments. Gene transcripts (12,730) with a normalized read count mean ≥ 5 were divided into 9 categories by magnitude of read counts (Table 1) for functional annotation analysis. The 13 most abundant transcripts were casein and whey proteins, regulators of milk fat synthesis and secretion, a ubiquitinating protein, and a tRNA transporter and together accounted for 67% of the 23M coding reads. Ingenuity pathway analysis revealed mTOR, JAK/STAT, PPARα, and ubiquitin proteasome pathways were enriched with reads ≥ 200. Genes with < 200 reads corresponded to tissue homeostasis and immune response. Some of the most abundant genes corresponded to maintenance of translation, protein turnover, and amino acid recycling suggesting their importance in supporting mammary function and milk synthesis.

55 Elevated circulating serotonin alters calcium metabolism in mid-late lactation dairy cows.

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Serotonin is an evolutionary conserved monoamine that modulates a variety of physiological functions. Of interest in the dairy cow is serotonin's action on calcium homeostasis due to the increased calcium demand that occurs during lactation. Understanding serotonin's mechanism, specifically at the level of the mammary gland, is critical to understanding the role of serotonin during lactation. Therefore, the objective of this study was to determine how intravenous infusion of 5-hydroxytryptophan (5-HTP) in mid-late lactation dairy cows altered calcium metabolism. Twelve multiparous Holstein cows were blocked by parity in a randomized complete block design. Cows were intravenously infused daily at a constant rate with (1) 1.5 mg/kg 5-HTP (n = 6) or (2) 0.9% saline (n = 6) for 3 consecutive days with sampling through 24h post termination of final infusion. Baseline mammary biopsies, blood and milk samples were taken one day before initiation of treatment. Additional mammary biopsies and blood samples were collected at 0, 8, and 24h post final intravenous treatment. Mammary tissues were analyzed via qPCR to evaluate calcium metabolism genes. Milk samples were taken each morning before infusion and the morning following final infusion. Circulating serotonin increased in 5-HTP infused cows ($P = 0.03$), while circulating calcium concentrations were decreased ($P = 0.03$) relative to control cows. Relative expression of plasma membrane calcium ATPase 2 and calcium release-activated channel protein 1 were upregulated in 5-HTP treated cows when compared with control cows ($P = 0.118$ and $P = 0.116$, respectively). Cows infused with 5-HTP had increased ($P = 0.01$) milk serotonin concentrations on d 4 and increased milk calcium concentration ($P = 0.03$) on d 3 of the experiment. Eight hours after final intravenous treatment, relative expression of parathyroid hormone-related peptide was increased ($P = 0.04$) and mammary gland serotonin content tended to be elevated ($P = 0.08$) relative to control. This supports previous rodent and cow data from our lab on serotonin's regulation of mammary gland physiology and calcium trafficking.

Key Words: serotonin, calcium

56 Methionine supplementation impacts casein gene expression and cell death in the mammary tissue of lactating dairy goats fed low and adequate net energy supplies.

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Methionine supplementation is known to modulate milk and protein yields in dairy ruminants. However, the mechanism involved in this phenomenon is still unknown. The cellular and molecular responses in the mammary tissue to methionine (Met) supplementation through HMBi (MetaSmart, Adisseo) at low (LE) and adequate levels (AE) of NEL were investigated according to a 2 × 2 factorial arrangement on 48 multiparous Alpine goats at mid lactation, assigned to a randomized block design in 4 groups during 5 weeks. Each goat was genotyped for CSN1S1 gene to balance each group according to the high or low genotypes for CSN1S1 gene. A fixed amount of hay was distributed per group (1.17 Mcal/kg DM) and a fixed amount of concentrates was distributed individually (1.72 vs. 1.83 Mcal/kg DM in LE and AE, respectively). Metabolizable methionine were 1.95 vs. 2.45% of metabolizable protein in unbalanced (LE and AE) vs. balanced (LEMET and AEMET) diets, respectively. Goats (23) were slaughtered after 5 weeks of treatment. Mammary tissues were collected for the analyses of milk protein mRNA levels by real time RT-PCR and apoptosis by immunohistochemistry using TUNEL assay. A variance analysis was performed with genotype, E, Met and E x Met, E x genotype, Met x genotype interactions as fixed effects and for milk, pre-treatment data yields was used as a covariate. Met supplementation increased milk protein yield (+6.5 g/d; $P = 0.03$). The treatments did no modify LALBA whereas an interaction E x Met ($P = 0.001$) were observed for both CSN3 and CSN1S1 mRNA levels showing 2-fold increases with Met supplementation in goats fed AE diet. The supplementation of Met reduced by 51% apoptosis rates in the mammary tissue ($P < 0.01$). Apoptosis rate was correlated with milk yield (−0.48; $P < 0.05$). These results suggest that Met increased casein gene expression at AE level and reduced cell death which could partly explain the positive effect of Met supplementation on milk synthesis in the goat mammary tissue.

Key Words: dairy goat, mammary tissue, amino acid supplementation

Production, Management, and the Environment

57 Effects of exposure to heat stress during late gestation on the daily time budget of nulliparous Holstein heifers. I. M. Toledo*, V. Ouellet, B. D. Davidson, G. E. Dahl, and J. Laporta, *University of Florida, Gainesville, FL.*

Exposure of dairy cows to heat stress negatively affects cow welfare and performance during all the phases of the lactation cycle. Negative effects include decreased milk yield, reduced immune status during the transition period, and altered natural behavior. While we understand heat stress responses in cows, one question that has yet to be answered is how late gestation heat stress affects the daily time budget of pregnant heifers. Automated monitoring devices (Nedap, Netherlands) were used to document the behavioral activity of heifers during pre- (final 60 d of gestation) and postpartum (first 60 d of lactation) periods. Twenty-five pregnant Holstein heifers were housed in a freestall barns and enrolled to heat stress (HT; shade; n = 13) or cooling (CL; shade, soakers and fans; n = 12) treatments prepartum. All heifers were cooled postpartum. Heifers received a leg tag, which measured daily lying time, number of steps, and standing bouts, and a neck tag which measured eating and rumination times. Heat stressed heifers tended to spend more time eating (224 vs 183 min/d; $P = 0.07$) and less time ruminating (465 vs 518min/d; $P = 0.05$) during the prepartum period compared with CL, but DMI did not differ. During the postpartum period, HT spent more time eating (209 vs 180 min/d; $P < 0.05$) during wk 1 to 4 of lactation, but no differences in rumination was observed ($P = 0.85$). Lying time was reduced ($P = 0.01$) by 59 and 88 min during weeks −7 and −6 prepartum and 84 and 50 min ($P = 0.05$) during wk 2 and 3 postpartum in HT. The number of steps was greater for HT during the postpartum period, from wk 2 to 9 (3038 vs 2653 steps/d; $P = 0.02$). Eating frequency was similar during pre- and postpartum ($P < 0.67$), however, HT had larger meals at night during the pre and postpartum periods compared with CL heifers. In summary, exposure to heat stress during late gestation affects the daily time budget of first lactation heifers during both the pre and postpartum periods. Insights onto heat stress effects in heifers may contribute to the development of more effective management strategies to mitigate heat load.

Key Words: heat stress, late gestation, behavioral activity

58 Metabolic and productive characteristics of sensitive and heat-tolerant phenotypes in dairy sheep. B. Chaalia, S. Serhan, S. Gonzalez-Luna, X. Such, A. A. K. Salama*, and G. Caja, *Research Group in Ruminants (G2R), Universitat Autonoma de Barcelona, Barcelona, Spain.*

The objective was to evaluate the effects of heat stress (HS) on Manchega dairy ewes differing in their phenotypical heat tolerance. Dairy ewes (n = 24) in late-lactation (158 ± 5 DIM) were submitted to a short-term (2 h) heat challenge (36°C, 43% humidity) in a temperature-controlled room. Rectal temperature (RT) and respiratory rate (RR) were recorded, and the ewes were classed by the RT and RR change ratio (CR = after/before HS). The CR values distributed normally and averaged 5.15 ± 0.22. Ten ewes differing in CR (Tolerant: A, 4.61 ± 0.24, n = 5; Sensitive: B, 5.69 ± 0.14, n = 5; $P < 0.01$) with similar milk yield were used in a crossover design of 2 periods (3 wk each) and 2 climatic conditions: 1) thermo-neutral (TN; 15 to 20°C day-night), and 2) HS (day, 37°C; night, 30°C). Humidity (50%) and dark-light (12–12 h) were constant. RT and RR, milk yield, and feed and water intakes were recorded daily. Milk and blood samples were collected and analyzed weekly. At d 19 of each period, ewes were submitted to glucose tolerance test (GTT; 0.25 g/kg BW) with blood samples collected at 10 time points (min ?15 to 120) for the analysis of glucose and insulin. Compared with TN, HS ewes experienced increased ($P < 0.001$) RT (+0.54°C), RR (+177%), water consumption (+35%), whereas feed intake decreased (−20%; $P < 0.001$). Milk yield (0.63 ± 0.05 kg/d) did not vary between treatments, but milk fat and milk protein contents decreased ($P < 0.01$) 14 and 17%, respectively in HS. The TN and HS ewes had similar blood glucose, insulin and NEFA, but HS had greater prolactin and creatinine values (+415 and 10%, respectively; $P < 0.01$). No differences were detected in RT, RR, feed intake, water consumption, milk yield, or milk composition between A and B phenotypes. Compared with TN, HS ewes had a similar insulin response to GTT ($P > 0.10$), but numerically greater ($P = 0.17$ to 0.19) glucose disposal. The A ewes under HS tended to have greater insulin secretion during GTT, but similar glucose disposal compared with B ewes. In conclusion, Manchega dairy ewes in were relatively tolerant to HS conditions, with few differences between A and B heat tolerance phenotypes.

Key Words: heat tolerance, metabolic indicators, dairy sheep

59 Pasture housing increases heat stress in late pregnant heifers: Management challenges for productivity and health. I. M. Toledo*, B. D. Davidson, V. Ouellet, G. E. Dahl, and J. Laporta, *University of Florida, Gainesville, FL.*

Exposure of dairy cows to heat stress during late gestation negatively impacts productivity and efficiency. Whereas cooling of dry cows is well accepted, late gestation heifers are often exposed to pasture management and thus heat stress. A better understanding of how heat stress affects the daily time budget of late gestation dairy heifers may aid in the management of these animals in adverse environmental conditions. Holstein heifers were either housed on pasture (PA; n = 6) or in a freestall barn and enrolled to heat stress (HT; shade; n = 6) or cooling (CL; shade, soakers and fans; n = 5) treatments. Automated monitoring devices (Nedap, Netherlands) were used to document behavioral activity. Heifers received a leg tag to measure daily lying time, number of steps and standing bouts and a neck tag to measure eating and rumination time. Temperature-humidity index (THI) and black globe temperature (BG) were recorded. Respiration rates (RR; breaths per min, bpm) were recorded thrice weekly for all groups. Average THI was 78 and BG was 29°C during the study period. RR were greater ($P < 0.01$) in PA and HT (96 and 61 bpm) compared with CL (48 bpm). Pasture heifers spent more time eating relative to HT and CL (270 ± 16.3 vs 163 ± 16.30 and 158 ± 18.0 min/d; $P < 0.01$) Rumination time was lower in PA compared with CL (357 ± 21.3 vs 438 ± 23.4 min/d; $P = 0.05$), but no difference was observed between PA and HT ($P = 0.48$). Pasture heifers spent less time lying down compared with CL and HT (582 ± 26.0 vs 837 ± 28.0 and 784 ± 24.2 min/d; $P < 0.01$). Furthermore, the number of steps was greater for PA compared with CL and HT heifers (5097 ± 184 vs 2060 ± 200 and 2159 ± 179 steps; $P < 0.01$). No difference in standing bouts was observed among the groups. In summary, exposure to heat stress during late gestation affects the daily time budget of pregnant heifers, especially if they are housed in pasture conditions. Insights onto heat stress effects on the daily time budget of late gestation heifers may contribute to the development of more effective management strategies to decrease the negative effects of heat exposure.

Key Words: lying time, eating time, behavioral activity

60 Methods for assessing chronic heat stress in dairy calves in a subtropical environment. V. Ouellet*, B. Dado-Senn, G. E. Dahl, and J. Laporta, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

Dairy calves are susceptible to heat stress as demonstrated by elevated physiological responses and reduced feed intake measured under high temperature and humidity. Yet methods to assess chronic heat stress have not been well-characterized in calves. Objectives were to evaluate the relationship between common environmental and animal-based indicators of heat stress in calves exposed to chronic heat stress or continuous cooling in a subtropical climate. Further, segmented regressions were performed to detect temperature-humidity index (THI) thresholds at which a change in physiological response was observed. Holstein calves were exposed to heat stress (HT, shade of barn, n = 24) or cooling (CL, shade of barn plus 2 fans, one at the calf level and one oscillating ~2 m above the ground; n = 24) from 2 to 42 d of age. Environmental (ambient temperature, humidity, THI, and wind speed) and animal-based (respiration and heart rate, RR, HR; rectal and skin temperature, RT, ST) indicators were recorded thrice daily whereas milk replacer and grain intake (MI, GI) were recorded daily from 15 to 42d of age. In both treatments, there was a positive correlation between most of the animal-based indicators and ambient temperature and THI, with the highest correlation obtained with ST ($r \geq 0.72$). Adding environmental indicators to regression equations to model animal-based indicators greatly improved the r^2 of the equations. Among all environmental indicators, THI obtained the highest r^2 when predicting rump ST ($r^2 \geq 0.92$). Segmented regression indicated that THI breakpoints at which RT and RR begin to rise and MI started to decrease under chronic heat stress were 67, 65, and 82 for RT, RR and MI respectively. Cooled calves had a threshold of 69 for RR but no breakpoints were identified for RT or MI, and ST variables in either treatment. In summary, our results suggest that ST is the optimal animal-based indicator to quantify heat load and THI is the best environmental indicator of heat stress in calves in a subtropical environment. At a practical level, heat mitigation should be applied when THI reaches 65 if calves are exposed to chronic heat stress.

Key Words: hyperthermia, correlation, threshold

61 Estimation of daily maximum temperature-humidity index thresholds affecting milk traits in Italian Brown Swiss. A. Maggiolino*[1], G. Dahl[2], N. Bartolomeo[3], A. Rossoni[4], and P. De Palo[1], [1]*Department of Veterinary Medicine, University of Bari "A. Moro," Bari, Italy*, [2]*Department of Animal Sciences, University of Florida*, *Gainesville, FL*, [3]*Department of Biomedical Science and Human Oncology, University of Bari "A. Moro," Bari, Italy*, [4]*Italian Brown Swiss Breeders Association*, *Verona, Italy*.

It is known that heat stress affects dairy cows' production and reproduction affecting both physiological and behavioral patterns. The aim of the work was to detect the temperature-humidity index (THI) thresholds, from the day of test-day sampling until 15 d before, for multiple milk production traits in Italian Brown Swiss dairy cows. A 10-yr data set (2009 – 2018) of test-day records was used. After the editing according to different criteria, the final set provided 202,776 test-day records of 23,296 Brown Swiss cows. All data were matched with the maximum daily THI calculated by temperature and humidity hourly recorded. First, a mixed linear model was fitted to obtain least squares estimates of THI effect on production traits. In a second set of analyses, the solutions for the THI class per parity class effect in the first model were used as the dependent variables to estimate change points in the relationship between production parameters and heat load, applying a 2-phase regression analysis. Results showed that there were no THI thresholds for milk yield in Brown Swiss, in all parities categories. On the contrary, FCM was affected by heat stress with average THI thresholds over 75. Protein yield and cheese production were affected by heat stress with average THI over 74. Brown Swiss showed higher thermal tolerance aptitude than Holstein cows. Heat stress negatively affect cow's performance, although in Brow Swiss it occurs at daily maximum THI higher 2 to 3 points more than Holstein cows. The lack of fitting of the 2-phase regression model on milk yield in Brown Swiss highlights a different metabolic pathway of this breed than Holstein during heath stress. With the raise of THI, Brown Swiss cows tended to produce the same amount of milk, but with a decreasing quality, mainly for protein yield, and then with a significant effect on cheese yield, the most important use of this milk. Further studies are necessary to estimate the heat tolerance genetic component that could and should be included in the selection procedures.

Key Words: heat stress, temperature-humidity index (THI), Brown Swiss

Ruminant Nutrition General

62 Meta-analysis to quantify the effect of chromium supplementation on production in dairy cows and how it is affected by Cr source and stage of lactation. Y. Roman-Garcia*[1], D. Kleinschmit[2], and L. Moraes[1], [1]*The Ohio State University*, *Columbus, OH*, [2]*Zinpro Corporation*, *Eden Prairie, MN*.

The objective was to quantify the effect of Cr supplementation on production and how the effect changes based on Cr source and stage of lactation. A meta-analysis was performed with data from 28 studies and 93 treatment means from experiments testing the supplementation of Cr starting in the dry period (n = 38) and during lactation. Studies utilized Cr as Cr-Picolinate, Cr-Yeast, Cr-Chelate, Cr-Methionine, or Cr-Propionate. Using the metaphor package in the R software, we modeled the mean difference between the experimental (cows receiving Cr) and control groups. Response variables were milk yield (MY; kg/d), energy-corrected milk (ECM; kg/d), and dry matter intake (DMI; kg/d). Difference in MY had a quadratic response to Cr supplementation expressed in mg/d and a quadratic response to days in milk (DIM). The model $\Delta MY = –4.65 (\pm 1.85) + 0.640 (\pm 0.264) \times Cr – 0.0477 (\pm 0.0173) \times Cr^2 + 0.0949 (\pm 0.0318) \times DIM – 0.0005 (\pm 0.0002) \times DIM^2$ suggest supplementation of Cr is maximized at 6.7 mg and when cows are at 95 DIM. Maximum increase in MY is 2 kg/d and then continues at a decreasing rate up to 13 mg of Cr and in cows up to 156 DIM. Using Cr-methionine further increases MY by 2 kg/d but there was no difference with other sources of Cr. With the model for $\Delta ECM = –4.21 (\pm 1.65) + 0.697 (\pm 0.216) \times Cr – 0.0510 (\pm 0.0142) \times Cr^2 + 0.0843 (\pm 0.0310) \times DIM – 0.0004 (\pm 0.0004) \times DIM^2$ supplementation of 6.8 mg of Cr to cows up to 105 DIM is suggested for a maximum increase of 2.6 kg/d of ECM. Increase in ECM was not affected by Cr source. model $\Delta DMI = –3.19 (\pm 0.76) + 0.214 (\pm 0.099) \times Cr – 0.0113 (\pm 0.0066) \times Cr^2 + 0.0607 (\pm 0.0145) \times DIM – 0.0003 (\pm 0.0003) \times DIM^2$ suggest a dose of 9.5 mg of Cr up to 101 DIM to maximize DMI by 0.9 kg/d. DMI is increased another 2.9, 2.5, and 1 kg/d by Cr-Yeast, Cr-Methionine and Cr-Propionate respectively. Overall, the effect of Cr supplementation is affected by stage of lactation and Cr source. With these models and the cost of the different Cr products we can predict what stage of lactation, Cr amount, and sources would be most effective and give a recommendation that would maximize profits.

Key Words: chromium

63 Effect of supplementary source of selenium on animal performance during intramammary endotoxin challenge in lactating Holstein cows. K. M. Cruickshank*[1], B. Hatew[2], E. S. Ribeiro[1], and M. A. Steele[1], [1]*University of Guelph*, *Guelph, ON, Canada*, [2]*University of Alberta*, *Edmonton, AB, Canada*.

The objective of this experiment was to determine how source of supplementary selenium (Se) affects animal performance during an intramammary endotoxin challenge. Twenty mid-lactation multiparous Holstein cows (591 ± 46 kg BW) were blocked by days in milk (157 ± 17 DIM) and randomly assigned to 1 of 2 treatments: 1) 0.30 ppm (100% NRC requirements on a dry matter basis) of supplementary organic (selenized yeast) Se premix, or; 2) 0.30 ppm of supplementary inorganic (sodium selenite) Se premix, top dressed and mixed into a basal ration that was fed once daily. Dry matter intake (DMI) and milk production were recorded daily. Following a 75-d dietary adaptation period, cows received an intramammary infusion of 50 µg of lipopolysaccharides (LPS; *Escherichia coli* strain O111:B4) diluted in 10 mL of sterile saline in one front quarter. Rectal temperatures were recorded at −1.5, 0, 2, 4, 6, 8, 10, 12, 24, and 48 h. Data were analyzed using PROC GLIMMIX in SAS. Statistical models included the fixed effects of treatment, time, and their interaction, and the random effects of block and cow nested within treatment. During the adaptation period, average daily DMI (23.54 ± 0.483 kg) and milk production (34.97 ± 1.730 kg) were not different ($P > 0.05$) between treatments. Results revealed no treatment differences in daily milk production and DMI during the LPS challenge, although there was a time effect for all parameters ($P < 0.001$). There was a tendency ($P = 0.07$) for inorganic cows to have higher DMI in the 12 h following LPS infusions. Cows experienced a significant ($P < 0.001$) change in body temperature during the day of LPS infusions, with a peak temperature occurring 6 h following infusions (40.59 ± 0.107°C), with no differences between treatments. These results indicate that animal performance during immune challenge is minimally affected by source of supplementary Se. Further analysis is required to determine antioxidant capacity and oxidative stress in cows supplemented with differing sources of Se.

Key Words: trace mineral, immunity, nutrition

64 Impact of supplementary trace mineral source on health and reproduction in lactating dairy cows. B. Mion*[1], J. F. W. Spricigo[1], K. King[1], L. Ogilvie[1], O. Chiu[1], L. Lobe[1], B. Van Winters[1], E. Merry[1], S. LeBlanc[2], M. A. Steele[1], B. W. McBride[1], and E. S. Ribeiro[1], [1]*Department of Animal Biosciences, University of Guelph*, *Guelph, ON, Canada*, [2]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*.

Our objectives were to evaluate the impact of supplementary trace minerals source, inorganic (ITM; Co, Cu, Mn, Zn sulfates and sodium selenite) or organic (OTM; Co, Cu, Mn, Zn proteinates and Se yeast; Bioplex Sel-Plex, Alltech Inc.), fed at 100% of recommended levels, on the incidence of clinical disease and the intervals from calving to resumption of estrous cyclicity and to first AI. Heifers and cows (n = 240) were enrolled at 45 ± 3 d before expected calving date, blocked by parity and BCS, and allocated randomly to ITM or OTM supplementation. Automatic feeding gates were used to assign treatments to individual cows through 150 DIM. After calving, diagnoses of clinical diseases were performed by the research team and farm personnel. Starting on d 21 ± 3, ovarian structures were examined weekly by ultrasonography to determine the first appearance of a corpus luteum. Cows received injections of $PGF_{2?}$ on d 42 ± 3 and 56 ± 3, and estrus behavior was evaluated using activity monitors. Continuous data were analyzed by ANOVA and binary data were analyzed by logistic regression using the GLIMMIX procedure of SAS. Statistical models included the effects of treatment, parity, season, and their interactions. P values < 0.05 were considered statistical difference and P values < 0.1 were considered tendency. Analyses of data from 209 cows indicated that fewer cows in OTM group tended to have postpartum diseases than cows in ITM (ITM = 26.5 vs. OTM = 14.6%; $P = 0.07$). Cows in OTM group resumed postpartum estrous cyclicity earlier than cows in the ITM group (ITM = 5.3 ± 0.2 vs. OTM = 4.7 ± 0.2 wk; $P = 0.04$). OTM had a greater proportion of estrous cyclic cows than ITM in wk 4 (ITM = 39.2 vs. OTM 55.7%, $P = 0.03$) and 5 (ITM = 56.4 vs. OTM = 75.0%, $P = 0.01$), but not wk 6 (INO = 78.1 vs. OTM = 88.2%, $P = 0.09$) and later. No differences between treatments were observed in proportion of cows detected in estrus after the second $PGF_{2\alpha}$ (ITM = 65.2 vs. OTM = 64.6%, $P = 0.94$) or the interval from calving to first AI (ITM = 63.2 ± 0.9 vs. OTM = 64.4 ± 0.9 d; $P = 0.32$). Our results indicate that replacement of ITM by OTM in pre- and postpartum diets improved postpartum health and hastened resumption of estrous cyclicity.

Key Words: trace minerals, health, reproduction

65 Impact of supplementary trace mineral source on production, feed intake and efficiency, metabolism, and rumen parameters in dairy cows. B. Mion*[1], J. F. W. Spricigo[1], E. Cran[1], L. Ogilvie[1], K. King[1], S. Anan[1], D. Stratas[1], B. Smith[1], Y. Chen[2], L. L. Guan[2], T. J. DeVries[1], S. J. LeBlanc[3], M. A. Steele[1], B. W. McBride[1], E. S. Ribeiro[1], [1]*Department of Animal Biosciences, University of Guelph*, *Guelph. ON, Canada*, [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta*, *Edmonton, AB, Canada*, [3]*Department*

of Population Medicine, University of Guelph, Guelph, ON, Canada.

Our objectives were to evaluate the impact of supplementary trace minerals source, inorganic (ITM; Co, Cu, Mn, Zn sulfates and sodium selenite) or organic (OTM; Co, Cu, Mn, Zn proteinates and Se yeast; Bioplex Sel-Plex, Alltech Inc.), fed at 100% of recommended levels, on milk production, dry matter intake (DMI), gross feed efficiency, blood metabolites, rumen fluid pH and volatile fatty acid (VFA) concentration, and rumination activity. Heifers and cows (n = 240) were enrolled at 45 ± 3d before expected calving date, blocked by parity and BCS, and allocated randomly to ITM or OTM treatments. Cows in both groups were fed the same diet, except for the source of supplemental TM, using automatic feeding gates to assign treatments to individual cows, which were fed until 150 DIM. Blood was collected on d −4, 0, 3, 7, 10, 14, 23, and 65 relative to calving. Cows were fitted with a neck-based collar to measure rumination activity, and ruminal fluid was collected on d −21, 23 and 65 ± 3 relative to calving. Data were analyzed by ANOVA using PROC GLIMMIX of SAS. Statistical models included the effects of treatment, parity, season, time, and their interactions. For repeated measures, data were summarized weekly and cow nested within treatment was considered a random term. Analyses of data from 145 cows indicated differences in prepartum DMI (ITM = 12.3 ± 0.3 vs. OTM = 13.1 ± 0.3 kg/d; $P = 0.04$). Cows in OTM group had reduced concentration of NEFA (ITM = 0.55 ± 0.02 vs. OTM = 0.48 ± 0.02 mmol/L; $P < 0.01$), and greater concentration of albumin (ITM = 35.29 ± 0.21 vs. OTM = 35.89 ± 0.21 g/L; $P = 0.04$) in serum compared with cows in ITM group. Cows in OTM tended to have greater concentration of butyric acid (ITM = 9.4 ± 0.5 vs. OTM = 10.7 ± 0.5 μmol/mL; $P = 0.06$), valeric acid (ITM = 1.5 ± 0.07 vs. OTM = 1.7 ± 0.07 μmol/mL; $P = 0.06$) and total VFA (ITM = 96.5 ± 2.7 vs. OTM = 102.7 ± 2.7 μmol/mL; $P = 0.10$) in ruminal fluid on d 23 than cows in ITM. Cows in ITM spent more time ruminating during the prepartum (ITM = 480 ± 13 vs. OTM = 442 ± 13 min/d; $P = 0.04$) and postpartum periods (ITM = 468 ± 11 vs. OTM = 430 ± 11 min/d; $P = 0.01$. Postpartum DMI, yields of milk, energy-corrected milk, protein, and fat, gross feed efficiency, and ruminal fluid pH did not differ between treatment ($P > 0.1$). Our results indicate that replacement of inorganic sources of supplementary TM by organic sources affects prepartum feed intake and rumen parameters, and slightly improve the postpartum metabolism of dairy cows.

Key Words: trace minerals, feed intake, milk production

66 Controlled trial of the effect of negative dietary cation-anion difference on postpartum health and culling of dairy cows.

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The objective of this study was to assess the effects of a negative dietary cation-anion difference (DCAD) dry cow diet on postpartum health and culling. Cows from 4 commercial dairy farms in Ontario, Canada were enrolled in a randomized controlled trial from November 2017 to April 2019. Close-up pens (1 per farm) with cows 3 wk before expected calving were randomly assigned to a negative DCAD (TRT; −100 mEq/kg DM; target urine pH 6.0–6.5) or a control diet (CON; +95 mEq/kg DM with a placebo supplement). Each pen was fed TRT or CON for 3 mo (one period) then switched to the other treatment for the next period, with 4 periods per farm. Body condition score (BCS) was measured at enrollment and urine pH was measured weekly until calving. Data from 1086 animals (TRT: n = 681; CON: n = 405) that received the assigned diet for >2 wk were included. The incidence of milk fever (MF), retained placenta (RP), metritis, ketosis (blood BHB >1.2 mmol/L, measured weekly in wk 1 and 2), clinical mastitis <30 DIM (CM), displaced abomasum (DA), purulent vaginal discharge (PVD, assessed once at wk 5), ≥ 1 disease (DIS) or culling by 35 DIM were analyzed with logistic regression models with treatment, parity, BCS, and their interactions, accounting for pen-level randomization and clustering of animals within farm with random effects. There were no interactions of treatment with parity or BCS for any outcome. There were only 43 cases of MF, allowing only univariable analysis. The incidence of MF was 5 ± 3% in CON and 1 ± 1% in TRT ($P = 0.18$). There were no treatment effects (CON vs TRT, LSM ± SE) on RP (7 ± 3%; 6 ± 2%; $P = 0.71$), metritis (11 vs 12%; SE = 4; $P = 0.83$), ketosis (21 vs 23%; SE = 4; $P = 0.59$, PVD (13 vs 12%; SE = 3; $P = 0.51$), or DIS (44 vs 41%; SE = 7; $P = 0.41$). Cows fed TRT had lesser incidence of CM (4 vs 2%; SE = 1, $P = 0.09$) and DA (3 vs 1%; SE = 1, $P = 0.05$). Culling <35 DIM tended to be greater in CON (7 ± 2%) than TRT (5 ± 1%, $P = 0.11$). Under commercial herd conditions, a negative DCAD fed 3 wk before parturition improved some but not all health outcomes assessed.

Key Words: milk fever, transition cow, nutrition

67 Impact of supplementary trace mineral source on immune cell function of dairy cows and its association with postpartum diseases.

L. Ogilvie*[1], J. F. W. Spricigo[1], B. Mion[1], B. Van Winters[1], M. A. Steele[1], B. W. McBride[1], S. J. LeBlanc[2], and E. S. Ribeiro[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Our objectives were to evaluate the impact of supplementary trace minerals source, inorganic (ITM; Co, Cu, Mn, Zn sulfates and sodium selenite) or organic (OTM; Co, Cu, Mn, Zn proteinates and Se yeast; Bioplex Sel-Plex, Alltech Inc.), fed at 100% of recommended levels, on neutrophil function in vitro and their association with postpartum disease. Heifers and cows (n = 240) were enrolled at 45 ± 3 d before expected calving date, blocked by parity and BCS, and allocated randomly to ITM or OTM supplementation. Cows in both treatments were fed the same diet, except for the source of supplementary TM. Automatic feeding gates were used to assign treatments to individual cows. Blood was collected on days −10 ± 3 and 7 ± 3 relative to calving in a subgroup of cows (n = 104) to measure neutrophil phagocytic capacity in vitro using fluorescence-labeled beads and flow cytometry. Diagnoses of clinical diseases were performed by the research team and farm personnel. To evaluate the association of in vitro assay responses with the incidence of postpartum disease, cows were categorized as low response (below median) or high response (above median). Continuous data were analyzed by ANOVA and binary data were analyzed by logistic regression using the GLIMMIX procedure of SAS. Statistical models included the fixed effects of treatment, parity, season, and their interactions. Treatment did not affect ($P > 0.60$) the percentage of neutrophils performing phagocytosis on d −10 or 7, which averaged 24.6 and 23.9% respectively. However, the mean fluorescence intensity of phagocytosis on d 7 was greater for OTM than ITM (SQRT scale: 91 vs. 85 ± 1.8, respectively; $P = 0.03$). Cows classified as low prepartum phagocytosis intensity tended to have greater incidence of postpartum diseases (low = 33.3 vs. high = 15.3%; $P = 0.06$). In conclusion, replacement of inorganic sources of supplementary TM by organic sources modestly improved one measure of phagocytic capacity of neutrophils in vitro. In addition, phagocytosis intensity response of our in vitro assay was negatively associated with incidence of postpartum diseases.

Key Words: trace minerals, immunity, health

Animal Health: Transition Cow 2

68 Relationship between nutrient metabolism during the periparturient period and health measures in a Pacific Northwest dairy herd. C. Y. Tsai*[1], H. H. Hung[1], T. Weber[1], W. J. Price[2], and P. Rezamand[1], [1]*Department of Animal and Veterinary Science, University of Idaho*, *Moscow, ID*, [2]*Statistical Programs, College of Agricultural and Life Sciences, University of Idaho*, *Moscow, ID.*

During the periparturient period, dairy cows mobilize stored nutrients to support fetal development and milk production. The objective of the present study was to determine the relationship between the serum lipid-soluble vitamins of dairy cows and calves with health status of the cows during the periparturient period. Blood samples of cows were obtained from early Spring to Winter of 2018 for a total of 645 periparturient cows on d −21, d −7, d 1, d 7 and d 14 relative to calving. Blood samples of calves were obtained (n = 459) within the first 4 d of life. Sera were analyzed for lipid-soluble vitamins (α-tocopherol, β-carotene, and retinol) via HPLC. Health records were categorized based on the occurrence of postpartum diseases (lameness, mastitis, pneumonia). The data were analyzed using linear mixed models and correlations in SAS with significance declared at $P \leq 0.05$ and tendency when $P < 0.1$. Results showed that cows with mastitis had greater serum retinol compared with that of healthy cows postpartum ($P < 0.01$). There was some evidence of a serum α-tocopherol time × mastitis interaction ($P = 0.08$). There was a significant interaction between the time relative to calving and season on serum α-tocopherol, β-carotene, and retinol concentration regardless of health status ($P < 0.001$). For mastitic cows, there were negative correlations for serum retinol and β-carotene between cows and calves on d 14 postpartum. In addition, negative correlations were observed between cows and calves for lameness (β-carotene) and pneumonia (α-tocopherol) on d 14 postpartum. In summary, disease affects the lipid soluble vitamins status of periparturient cows, and consequently calves may experience health issues.

Key Words: lipid-soluble vitamin, health status, dairy cow

69 Hepatic differential gene expression of cows clustered by postpartum metabolites: A model for susceptibility to lipid-related metabolic disorders. R. Pralle*[1], W. Li[2], and H. White[1], [1]*Department of Dairy Science, University of Wisconsin*, *Madison, WI*, [2]*Cell Wall Biology and Utilization Laboratory, USDA-ARS*, *Madison, WI.*

We propose that cows developing metabolic disorders (MD) in the absence of induction, compared with those that do not, represent groups of MD susceptibility. Our study objectives were to identify differentially expressed genes (DEG) and enriched metabolic pathways (EMP) associated with susceptibility to MD. From a larger multiparous transition cow study, the control treatment (n = 13) was clustered (K-means, R, v. 3.5.2) based on postpartum blood fatty acid, BHB, and liver triglyceride concentrations. Metabolite means within the largest 2 clusters revealed one cluster with a preferable low metabolite concentration profile (less susceptible, LS) and a cluster with disfavorably greater metabolite concentrations (MD susceptible, MS). From each cluster, 3 cows were selected for whole transcriptome RNaseq of liver tissue collected at +1 and +14 d relative to calving (DRTC). Alignment of reads to the *Bos taurus* genome, DEG analysis between LS and MS clusters within DRTC, and EMP analysis were done using STAR (v.2.5.2b), Cufflinks (Cuffdiff, v.2.2.1), and DAVID (v.6.8), respectively. DEG and EMP were considered significant and tendencies when $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively, after FDR-adjustment. Within +1 DRTC, 102 significant and 35 DEG tendencies were observed. Unique genes with an absolute fold change (FC) > 5.2 were *REC8*, *IFI6*, and *IFI27*. There were 175 significant and 48 DEG tendencies on +14 DRTC; unique genes (FC > 8) included *MEDAG*, *FABP4*, *HP*, and *SAA2*. Between DRTC, 44 DEG with $P \leq 0.10$ were shared, including *ULBP27*, *SFRP2*, and *PDYN*. Unique +1 DRTC EMP included protein processing by the endoplasmic reticulum, RIG-I-like receptor signaling pathway, and phagosome. Protein digestion and absorption, regulation of lipolysis in adipocytes, and PPAR signaling pathway were unique EMP to +14 DRTC. Four EMP were shared between DRTC including glutathione metabolism and cytochrome P450 metabolism of drugs and xenobiotics. These results suggest hepatic transcriptome features of MD susceptibility relate to immunity, inflammation, regulation of lipid metabolism, and P450-mediated metabolism.

Key Words: ketosis, fatty liver, RNAseq

70 Effect of metabolic and digestive disorders on patterns of behavioral, physiological, and performance parameters of lactating dairy cows. M. M. Perez*[1], E. M. Cabrera[1], C. Rial[1], D. V. Nydam[2], and J. O. Giordano[1], [1]*Department of Animal Science, Cornell University*, *Ithaca, NY*, [2]*Department of Population Medicine and Diagnostic Sciences, Cornell University*, *Ithaca, NY.*

Our objective was to characterize the pattern of multiple behavioral, physiological, and performance parameters collected by sensors in Holstein cows diagnosed with displaced abomasum (DA), clinical ketosis (KET) and indigestion (IND). Sensor parameters included activity (ACT), resting (RET), rumination (RUM) and eating (EAT) behavior, reticulo-rumen temperature (TEMP), milk production (MILK), milk components (FAT; PROT = protein), milk conductivity (CON), and BW. Cows were clinically examined daily until 28 DIM. Sensor data from d −7 to 7 relative to clinical diagnosis was compared for cows with DA (DAO = 5) or KET and IND only (K-IO = 67), cows with DA or KET and IND plus another disorder during the 15 d period (DA+ = 40, K-I+ = 86), and cows with no clinical signs of disease (NCD n = 824). Data were analyzed by ANOVA with repeated measurements with group, time, and their interaction, and lactation (1, 2, 3+) as fixed effects. Cow within group was a random effect and the subject of repeated measures. Cows in DAO, DA+, K-IO and K-I+ had lesser ($P < 0.01$) RUM (d −7 to 7 for DA+ and K-I+, d −7 to −6 for DAO and K-IO), lesser ($P < 0.01$) EAT (d −7 to 7 for DA+, d −6 to 7 for DAO, d −4 to 7 for K-I+ and d −3 to 7 for K-IO), and lesser ($P < 0.01$) MILK (d −7 to 7 for DAO and DA+, d −7 to 7 for K-I+ and d −6 to 7 for K-IO) than cows in NCD. Compared with cows in NCD, DAO and DA+ had more ($P < 0.01$) RET from d −7 to 7 and d −4 to −1, respectively but then less ($P < 0.01$) RET from d 3 to 7. For cows in K-I+ and K-IO RET was less than for cows in NCD from d −4 to 2 and from d −3 to −1, respectively. FAT and FAT:PROT ratio was greater ($P < 0.01$) for K-IO and KI+ from d −2 to 7. Compared with cows in NCD, FAT and FAT:PROT was greater ($P < 0.01$) from d −6 to 7 for cows DA+ whereas for cows in DAO it was greater from d −1 to 7 . Cows in DA+ lost more BW than NCD cows from d −3 to −1. TEMP was less ($P < 0.01$) for cows DAO and DA+ than NCD from d −7 to d 0. We conclude that cows with metabolic and digestive disorders had specific patterns of change in behavioral, physiological and productive parameters monitored by sensors.

Key Words: sensor, disease, dairy cow

71 Antimicrobial resistance in fecal commensal bacteria isolated from dairy cows in California. E. Abdelfattah*[1], P. Ekong[1], E. Okello[1,2], T. Chamchoy[1], B. Karle[3], R. Black[4], D. Sheedy[1], W. El-Ashmawy[1], D. Williams[1], D. Califano[1], L. Duran[1], J. Ongom[1], B. Byrne[5], T. Lehenbauer[1,2], S. Aly[1,2], [1]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California*, *Tulare, CA*, [2]*Department of Population Health & Reproduction, School of Veterinary Medicine, University of California*, *Davis, CA*, [3]*Cooperative Extension, Division of Agriculture and Natural Resources, University of California*, *Orland, CA*, [4]*Cooperative Extension, Division of Agriculture and Natural Resources, University of California*, *Santa Rosa, CA*, [5]*Department of Pathology, Microbiology, and Immunolo-*

gy, School of Veterinary Medicine, University of California, *Davis, CA*.

The objective of study was to describe antimicrobial resistance (AMR) patterns in *Escherichia coli* and *Enterococcus* spp. isolated from adult cows, following the implementation of Senate Bill 27. The 10 study dairies were distributed across California's 3 milk sheds: Northern CA (NCA), Northern San Joaquin Valley (NSJV) and the Greater Southern California (GSCA). Individual cow fecal samples were collected monthly from pre-partum to 120 d in milk from 2 cohorts each of 12 cows enrolled on each study dairy during the winter and summer. Isolated *E. coli* and *Enterococcus* spp. were tested for antimicrobial susceptibility to determine the minimum inhibitory concentration. Antimicrobial susceptibility testing was completed on 2,171 *E. coli* and 2,158 *Enterococcus* isolates. The proportion of AMR was calculated in Stata with a P-value < 0.05 was the threshold of significance. None of the *E. coli* isolates and 37.3% of *Enterococcus* isolates were pan susceptible to all drug classes tested. The *E. coli* AMR to tilmicosin (99.9%), tylosin (99.8%), tiamulin (98.9%), florfenicol (83.3%) gamithromycin (74.0%), and tildipirosin (20.51%) was common, while *E. coli* AMR to ampicillin (1.1%), ceftiofur (1.9%), danofloxacin (4.0%), enrofloxacin (3.3%), gentamicin (0.3%), and neomycin (1.6%) was rare. Like *E. coli*, *Enterococcus* spp. were highly resistant to tildipirosin (50%), tilmicosin (48%), tiamulin (42%), and florfenicol (46%). In addition, enterococci showed a lower rate of resistance to ampicillin (0.23%), and penicillin (0.20%). Multidrug resistance (MDR) (resistance to ≥3 antimicrobial classes) was observed in 38% of *E. coli* isolates, and 39% of *Enterococcus* spp. isolates recovered during the winter season showed higher MDR prevalence compared with the summer isolates (P < 0.01). A higher prevalence of MDR was observed in NSJV and GSCA compared with NCA (P < 0.01). Our findings show high rates of AMR to drugs commonly administered to calves. Conversely, very low resistance was observed for drugs used for adult dairy cows such as cephalosporins, and penicillin. Overall, our findings identified important differences in AMR by antimicrobial classes, region and season.

Key Words: antimicrobial resistance, cattle

72 Performance of the machine learning method XGBoost for prediction of clinical health disorders in lactating dairy cows.

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Our objective was to evaluate the ability of the machine learning method XGBoost to predict the occurrence of different clinical health disorders experienced by lactating dairy cows in early lactation using multiple sensor and non-sensor data. The clinical health status of lactating Holstein cows (n = 1,211) was determined based on daily clinical examination from 1 to 30 DIM. Clinical conditions recorded were: metritis, mastitis, ketosis, indigestion, and displaced abomasum. Cows were considered to have a clinical disorder for all days at which any of these conditions were recorded. Sensor data offered to ML models were: physical activity, resting behavior, reticulo-rumen temperature, rumination, eating behavior, and environmental temperature and humidity from −21 to 30 DIM. After calving data was also available for BW and daily milk volume, conductivity, and components (fat, protein, lactose). Non-sensor data used were: previous health and reproductive events, production records, and stocking density. For each individual (metritis, mastitis) or group of disorders (Met-Dig: ketosis, displaced abomasum, indigestion) of interest, an XGBoost model was developed using 80% of the data for training and 20% for testing. Performance metrics for models for disorders of interest were estimated (Table 1). Some models (mastitis and Met-Dig) tended to overfit the training data and were not able to generalize to the testing data, likely due to the limited training outcomes and unbalanced ratio of positive to negative outcomes. Machine learning models created using XGBoost had a different performance for predicting different health disorders when offered multiple cow behavioral, physiological, and performance sensor parameters, environmental sensor data, and health, reproductive, and performance records.

Table 1 (Abstr. 72). Performance metrics (sensitivity, Se; specificity, Sp) for models of disorders of interest

	Train set		Test set	
Disorder	Se	Sp	Se	Sp
Mastitis	99.2	96.8	68.7	95.8
Metritis	99.5	90.5	90.0	88.4
Met-Dig	100.0	93.4	75.0	92.1

Key Words: machine-learning, disease, dairy cow

73 Performance of different machine learning methods for prediction of the health status of lactating dairy cows.

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Our objective was to evaluate the performance of different machine learning methods (ML) to predict the health status of dairy cows based on multiple sensor and non-sensor data. The clinical health status (clinical or no clinical disease) of lactating Holstein cows (n = 1,211) was determined based on daily clinical examination from 1 to 30 DIM. Disorders recorded were: metritis, mastitis, ketosis, indigestion, displaced abomasum, and pneumonia. Cows were considered to have a clinical disorder for all days at which any of these conditions were recorded. Sensor data offered to ML models were: physical activity, resting behavior, reticulo-rumen temperature, rumination, eating behavior, and environmental temperature and humidity from −21 to 30 DIM. After calving data was also available for BW and daily milk volume, conductivity, and components (fat, protein, lactose). Non-sensor data used were: previous health and reproductive events, production records, and pen stocking density. Models created and evaluated using Python included: XGBoost, Multi-Layer Perceptron (MLP) and Recurrent Neural Network (RNN). Data available was split to use 80% for training and 20% for testing. Sensitivity (Se) and specificity (Sp) for predicting the occurrence of clinical health disorders were estimated. For MLP Se and Sp for the training set were 100% and 98% and for testing set were 43% and 96%. For RNN Se and Sp for the training set were 99% and 67% and for testing set were 70% and 67%. For XGBoost Se and Sp for the training set were 95% and 90% and for testing set were 88% and 88%. Models for MLP and RNN tended to overfit the training data and were not able to generalize to the testing data, likely due to the limited training outcomes and unbalanced ratio of positive to negative outcomes. Thus, some predictive models created with ML methods may be effective for predicting the health status of cows when including multiple cow behavioral, physiological, and performance sensor parameters, environmental sensor data, and health, reproductive, and performance records.

Key Words: prediction, disease, dairy cow

Growth and Development

74 Source of cobalt fed during late pregnancy to Holstein cows affects postnatal calf growth and innate immune function. A. S. Alharthi*[1,2], E. Abdel-Hamied[3], H. Dai[4], Y. Liang[1], V. Lopreiato[5], A. A. Elolimy[1,6], M. T. Socha[7], and J. J. Loor[1], [1]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois*, *Urbana, IL*, [2]*Department of Animal Production, College of Food and Agriculture Sciences, King Saud University*, *Riyadh, Saudi Arabia*, [3]*Animal medicine department, Beni-Suef University*, *Beni-Suef, Egypt*, [4]*College of Veterinary Medicine, Nanjing Agricultural University*, *Nanjing, PR China*, [5]*Department of Animal Sciences, Food and Nutrition Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore*, *Piacenza, Italy*, [6]*Department of Animal Production, National Research Centre*, *Dokki, Giza, Egypt*, [7]*Zinpro Corporation*, *Eden Prairie, MN*.

Maternal nutrition has a key role on fetal growth can program the long-term physiology of the offspring. We investigated effects of feeding 72 multiparous Holstein cows during late-pregnancy with an experimental source of Folic acid (FOA; Zinpro Corp.), 2 Co sources [Co glucoheptonate (Copro) or Co pectin (CoPectin), Zinpro Corp.] and rumen-protected Met (RPM) on calf developmental parameters at birth and through 9-wk of age. Cows were fed a basal diet [1.37 Mcal/kg of dry matter (DM), 14.5% crude protein] and randomly assigned (17–21 per treatment) to Copro, FOA+Copro, FOA+CoPectin, or FOA+CoPectin+RPM. In each FOA group cows received 50 g FOA. Co treatments delivered 1 ppm Co/kg DM. The RPM was fed at 0.09% of DMI to achieve a ratio of 2.8:1 Lys:Met in the MP. Calves received 3.8 L of first-milking colostrum from their dam within 6 h, and were housed in individual outdoor hutches bedded with straw, fed twice daily with a milk replacer, and had ad libitum access to a starter grain mix from birth to weaning at 42 d of age. Body weight, hip and wither height, hip width and body length were measured at birth and weekly through weaning (42 d of age) and until 9 wk of age. The statistical model in SAS 9.4 included treatment, age and their interactions as fixed effects. Preplanned contrasts were FOA vs. no FOA, CoPectin vs. Copro, and RPM vs. no RPM. Metabolite concentrations in plasma indicated no differences over time due to maternal diet in energy metabolism, liver function, or oxidant status. However, blood neutrophil phagocytosis capacity was greater ($P = 0.03$) in calves born to cows supplemented CoPectin. Although maternal diet did not alter developmental parameters at birth, during the first 9 wk of age calves born to cows supplemented CoPectin had greater ($P < 0.05$) starter intake and weekly average daily gain. Overall, data indicated that CoPectin supplementation during late-gestation had a positive effect on neonatal calf performance. Whether effects arose from greater availability in utero of vitamin B_{12} synthesized from Co by rumen microbes remains to be determined.

Key Words: fetal programming, vitamin B_{12}, epigenetics

75 The development of the fecal microbiome in Holstein dairy calves throughout the weaning transition. M. Hennessy*[1], B. Vecchiarelli[1], N. Indugu[1], J. Bender[1], C. Pappalardo[1], M. Leibstein[2], J. Toth[1], A. Katepalli[3], S. Garapati[4], and D. Pitta[1], [1]*University of Pennsylvania School of Veterinary Medicine*, *New Bolton Center Kennett Square, PA*, [2]*Oceanside High School*, *Oceanside, NY*, [3]*Northwest High School*, *Germantown, MD*, [4]*Drexel University*, *Philadelphia, PA*.

Calf diarrhea is the leading cause of death in pre-weaned calves and causes major economic losses to producers. A better understanding of the development of the microbiome throughout the weaning transition could lead to better treatment and prevention strategies. In this study, fecal samples were obtained from 10 cohabiting female Holstein dairy calves at 6 time points between age 2–4 weeks and age 12–14 weeks. Calves were fed on acidified milk until weaning at about 8 weeks of age and had access to starter grain throughout the course of the study. Fecal samples were extracted for genomic DNA, PCR-amplified for the V1-V2 region of the 16S rDNA bacteria gene, sequenced on the Illumina MiSeq platform, and analyzed using the QIIME2 pipeline. Bacterial richness, estimated by number of observed species, and bacterial diversity, estimated by Shannon diversity index, both differed significantly between time points and both increased over time ($P < 0.05$), with the largest increases occurring during the weaning transition. Both weighted and unweighted Unifrac analysis showed significant differences ($P < 0.05$) between bacterial communities across time points. Throughout the course of the study, Firmicutes was the dominant phylum, followed by Bacteroidetes. Seven bacterial genera were found to be significantly influenced by time: *Faecalibacterium, Ruminococcaceae, Prevotella, Clostridiales, Sutterella, Lachnospiraceae,* and *Coriobacteriaceae. Ruminococcaceae* was the most prevalent genus at TP1, 3, 5, and 6, *Bacteroides* was the most prevalent genus at TP2, and *Prevotella* was the most prevalent genus at TP4. Considerable variation was seen in the proportions of abundant genera between calves as well as between time points; however variations in phylogenetic makeup between individual calves decreased as the calves aged, indicating that cohabitation led the microbiota of individual calves to become more similar with time. Our results indicate that there is considerable flux in the calf microbiome throughout the weaning transition and add to our understanding of the progression of the microbiome during calf development.

Key Words: acidified milk, calf diarrhea, calf weaning

76 Early life indicators of future growth in Holstein dairy heifers. T. S. Steckler*[1], N. Lopez-Villalobos[2], and J. P. Boerman[1], [1]*Purdue University Department of Animal Sciences*, *West Lafayette, IN*, [2]*School of Agriculture and Environment, Massey University*, *Palmerston North, New Zealand*.

The objective of this study was to evaluate the long-term effects that early life (from 0 to 60 d of age) has on BW of growing heifers up to 400 d of age. Daily milk consumption, serum total protein, pneumonia and scours incidences, genetic body size, birthweights, and incremental BW were collected on a commercial dairy farm from October 1, 2015 to January 1, 2019. Holstein calves (n = 9,099) were fed whole pasteurized milk with a 5% fat and 30% protein enhancer added at 20 g per L of milk through a Förster-Technik automated calf feeding system (feeders = 8) for 60 d (range 48 – 126d). Calves were weighed at birth and several other times before calving. Average birth weight of calves was 40.6 ± 4.9 kg (mean ± SD), serum total protein was 6.7 ± 0.63 mg/dL, and cumulative 60 d milk consumption was 508.1 ± 67.3 L with a range of 179.9 to 785.1 L. Daily BWs were predicted for individual animals using third order orthogonal polynomial to model growth curves. The linear and quadratic effects of cumulative 60 d milk consumption, birthweight, feeder, year born, season born, respiratory incidence, and genetic body size score were significant ($P < 0.0001$) when predicting heifer BW at 400 d (pBW_{400}) of age ($R^2 = 0.31$). There was up to a 263 kg difference in pBW_{400} between the heaviest and lightest animal. Birthweight had a significant effect on predicted weights up to 400 d ($P < 0.001$), and for every 1 kg increase in birthweight, there was a 2.5 kg increase in pBW_{400}. Quadratic effect of cumulative 60 d milk consumption was significant up to 400 d ($P < 0.0001$). We divided 60 d milk consumption into quartiles. Heifers had the highest pBW_{400} in the third quartile, when 60 d consumption was between 507.8 and 552.5 L. Genetic body size showed a 21.5 kg difference in pBW_{400} between the top and bottom 25th percentile of heifers. Heifers were 4.2 kg lighter at 400 d if treated for respiratory disease 3+ times during the first 60 d of life, compared with heifers not treated for respiratory disease. Early life events continue to influence heifer growth up to 400 d of age.

Key Words: autofeeder, growth, milk consumption

77 Maternal body condition during late-pregnancy and its influence on calf development and whole-blood mRNA abundance after ex vivo lipopolysaccharide challenge. M. G. Lopes*[1,2], A. S. Alharthi[2,3], Y. Liang[2], H. Dai[4], V. Lopreiato[5], M. N. Corrêa[1], and J. J. Loor[2], [1]*NUPEEC (Núcleo de Pesquisa, Ensino e Extensão em Pecuária), Departamento de Clínicas Veterinária, Programa de Pós-Graduação em Biotecnologia, Universidade Federal de Pelotas*, *Pelotas, RS, Brazil*, [2]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois*, *Urbana, IL*, [3]*Department of Animal Production, College of Food and Agriculture Sciences, King Saud University*, *Riyadh, Saudi Arabia*, [4]*College of Veterinary Medicine, Nanjing Agricultural University*, *Nanjing, China*, [5]*Department of Animal Sciences, Food and Nutrition, Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore*, *Piacenza, Italy*.

Body reserves of dairy cows in late-pregnancy are directly related to energy status and play a key role in the partition of nutrients to the rapidly-growing fetus. We investigated effects of maternal body condition score (BCS) during late-pregnancy on calf development and whole blood mRNA abundance after ex vivo lipopolysaccharide (LPS) challenge. Forty-nine multiparous Holstein cows from a larger cohort were retrospectively divided by prepartal BCS into normal BCS ≤ 3.25 ($n = 30$; NormBCS) and high BCS ≥ 3.75 ($n = 19$; HighBCS) groups. Calf development was assessed through BW, wither and hip height, hip width and body length from birth to 9 wk of age. Blood samples were collected at birth (before receiving colostrum, d 0), and at 21 and 42 (at weaning) days of age for whole blood challenge with 3 μg/mL of LPS before RNA isolation. The statistical model used in SAS 9.4 included BCS, Age and their interactions as fixed effects. A One-Way ANOVA was used to determine differences in body measurements at birth with BCS as fixed effect. Birth BW was lower ($P = 0.03$) in calves born to HighBCS cows and remained lower ($P = 0.04$) at 9 wk of age. At birth, whole blood from calves born to HighBCS vs. NormBCS cows had greater ($P \leq 0.03$) mRNA abundance of *IL1B* and *GSR* coupled with lower *GPX1* after LPS challenge. The longitudinal analyses of d 0, 21, and 42 data revealed a BCS x Age effect for *GPX1* ($P = 0.02$) due to lower abundance at birth in blood from calves born to HighBCS vs. NormBCS cows. With the exception of *IRAK1*, *MPO*, *GSS*, and *CBS*, all genes experienced a decrease ($P < 0.01$) in abundance over time. Overall, results suggest that BCS in late-prepartum not only influences calf development in utero, but also the abundance of key genes related to cytokine production and antioxidant function in whole blood.

Key Words: BCS, fetal programming, neonatal immunity

78 Supplementation with cobalt sources, folic acid, and rumen-protected methionine during late pregnancy in Holstein cows alters whole-blood mRNA abundance after ex vivo lipopolysaccharide challenge in newborn calves. M. G. Lopes*[1,2], A. S. Alharthi[2,3], H. Dai[4], V. Lopreiato[5], E. Abdel-Hamied[6], M. N. Corrêa[1], M. T. Socha[7], and J. J. Loor[2], [1]*NUPEEC (Núcleo de Pesquisa, Ensino e Extensão em Pecuária), Departamento de Clínicas Veterinária, Programa de Pós-Graduação em Biotecnologia, Universidade Federal de Pelotas*, *Pelotas, RS, Brazil*, [2]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois*, *Urbana, IL*, [3]*Department of Animal Production, College of Food and Agriculture Sciences, King Saud University*, *Riyadh, Saudi Arabia*, [4]*College of Veterinary Medicine, Nanjing Agricultural University*, *Nanjing, China*, [5]*Department of Animal Sciences, Food and Nutrition, Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore*, *Piacenza, Italy*, [6]*Animal Medicine Department, Beni-Suef University*, *Beni-Suef, Egypt*, [7]*Zinpro Corporation*, *Eden Prairie, MN*.

An adequate supply of nutrients during fetal life is an important determinant of immunocompetence in the neonatal animal. We investigated effects of supplementing multiparous Holstein cows during the last 30-d of pregnancy with an experimental folic acid source (FOA), 2 Cobalt sources [Co glucoheptonate (Copro) or Co pectin (CoPectin), Zinpro Corp.] and rumen-protected Methionine (RPM) on calf whole blood mRNA abundance after ex vivo LPS challenge. Seventy-two cows individually fed a basal diet [1.37 Mcal/kg of DM, 14.5% CP] were randomly assigned (17–19 per diet) to Copro, FOA+Copro, FOA+CoPectin, or FOA+CoPectin+RPM until 30 d postpartum. Cows in each FOA group received 50 g FOA. Co treatments delivered 1 mg Co/kg DM. The RPM was fed at 0.09% of DM to achieve a ratio of 2.8:1 Lys:Met in the MP. Blood samples were collected at birth (before colostrum), 21 and 42 (weaning) d of age and immediately challenged with 3 μg/mL LPS. Target genes evaluated by RT-PCR are associated with immune response, antioxidant function, and 1-carbon metabolism. The statistical model used in SAS 9.4 included treatment, age and their interactions as fixed effects. Preplanned contrasts were FOA vs. no FOA, CoPectin vs. Copro, and RPM vs. no RPM. Abundance of *IRAK1* was the sole gene differentially expressed across all contrasts, a response associated with highest abundance in the FOA+CoPectin+RPM group. In addition to *IRAK1*, calves born from cows supplemented with FOA had greater ($P \leq 0.05$) abundance of *BPI* and *GPX1* coupled with lower *SELL*. Similarly, calves born from cows supplemented with CoPectin had greater ($P \leq 0.01$) *IRAK1* and lower ($P \leq 0.04$) *CXCR1*, *SELL*, and *MTR*. With the exception of *IRAK1*, *MPO*, *LYZ*, and *GSS*, all genes experienced a decrease ($P < 0.001$) in abundance over time. Overall, data suggest that greater maternal supply of nutrients associated with 1-carbon metabolism can modulate important pathways associated with immune response to a pathogen challenge in neonatal calves.

Key Words: epigenetics, nutritional programming, vitamin B_{12}

Lactation Biology

79 Laser capture microdissection-seq analysis of local and systemic responses of mammary epithelial cells in cows locally treated with lipopolysaccharide. R. K. Choudhary*[1], A. Spitzer[1], T. B. McFadden[2], E. M. Shangraw[2], R. O. Rodrigues[2], and F.-Q. Zhao[1], [1]*Department of Animal and Veterinary Sciences, University of Vermont*, *Burlington, VT*, [2]*Division of Animal Sciences, University of Missouri*, *Columbia, MO*.

We aimed to analyze transcriptional changes of mammary epithelial cells (MEC) isolated from bovine mammary glands after intramammary challenge with lipopolysaccharide (LPS). Ten multiparous cows were used in the study. Five treatment (T) and 5 control (C) cows were paired based on days in milk, milk yield and parity. For T cows, both mammary glands on one side of the udder were infused with LPS (50 µg in 10 mL saline); these glands were designated (TL). The contralateral glands received 10 mL saline and were designated (TS). Likewise, for C cows 2 ipsilateral glands received saline (CS) and the contralateral glands remained uninfused (CU). Mammary tissues were biopsied before (0 h) and at 3 and 12 h post-infusion and processed for laser capture microdissection (LCM). MEC were collected using LCM, total RNA was isolated and subjected to low-put RNA sequencing. Among various comparisons, we found 3167 (TL3 vs. TL0), 670 (TL12 vs. TL0), 2555 (TL3 vs. TS3), and 3823 (TL3 vs. CS3) differentially expressed genes [DEGs; FDR < 0.05, Log_2 (fold change) ≥ 1]. The major local responses of MEC in TL glands at 3h included upregulation of ribosome biogenesis, innate immunity and KEGG pathways of TNF, NOD-like receptor and NFKB signaling. Ingenuity pathway analysis showed activation of TNFR2, PI3/AKT, iNOS and acute phase protein response. Upstream regulators of these pathways predicted invasion of cell, chemotaxis and cell migration, and showed activated HIF1A network. Downregulated genes included network of carbohydrate metabolism, PPAR fatty acid biosynthesis, and several ionic transporters. Major systemic responses of MEC in TS glands showed weak cell mediated immune response, lymphocyte activation, and cytokine production responses. Ingenuity pathway analysis of systemic response genes at 3 h showed *p53* senescence pathway with activated upstream regulators as NFKB and TNFA. These results of comprehensive transcriptome profiling of MEC may explain gene regulation of local and systemic responses of MEC during *E. coli* mastitis.

Key Words: endotoxin, mastitis, RNA-sequencing

80 Sialylated oligosaccharide and sialic acid profile of colostrum, transition milk, and whole milk in primiparous and multiparous Holstein dairy cattle. A. J. Fischer-Tlustos*[1], R. K. H. Yoo[2], A. M. Garner[3], S. A. Bakker[2], S. M. Lyons[3], W. F. Zandberg[2], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph*, *Guelph, ON, Canada*, [2]*Department of Chemistry, University of British Columbia*, *Kelowna, BC, Canada*, [3]*Department of Biology, University of British Columbia*, *Kelowna, BC, Canada*.

The objective of this study was to characterize the sialylated oligosaccharide (OS) and sialic acid (SA) profile during the first week of lactation in primi- (PP) and multi-parous (MP) dairy cattle. The animal experiment consisted of MP (n = 10) and PP (n = 10) Holstein dairy cows assigned to the study at the time of calving. Colostrum (milking 1) and milk (milkings 2–4, 8, and 14) samples were collected twice daily. Oligosaccharides were quantified by capillary electrophoresis with laser-induced fluorescence (CE-LIF) detection and HPLC-MS/MS. Median OS levels were deemed statistically significant if 2-tailed Mann-Whitney U values were lower than U_{crit} at $P < 0.05$ or 0.01, while differences in OS levels between parity were assessed using a 2-tailed Student's t test with $P < 0.05$ considered significant. Total SA were quantified by HPLC-MS after cleavage from milk OS or glycoproteins by weak acid hydrolysis and evaluated using a Wilcoxon rank test with $P < 0.05$ considered significant. A total of 9 SA were quantitated, including 5-*N*-acetylneuraminic acid (Neu5Ac), *N*-glycolneuraminic acid (Neu5Gc), and 7 *O*-acetylated analogs; 2-keto-3-deoxy-D-glycero-D-galacto-nonulsonic acid (Kdn). A 4-fold decrease in concentrations of both OS- and glycoprotein-bound SA over the first 14 milkings was observed. Median levels of OS-bound Neu5Ac increased ($P < 0.001$) from 58 to 79% of total SA to the decrement of Neu5Gc (14 to 7%) and Kdn (28 to 17%) ($P < 0.001$). Cow parity influenced levels of specific SA species at milking 14 ($P < 0.005$), but not in colostrum ($P > 0.08$). The absolute levels of sialylated OS decreased ($P < 0.002$) from milking 1 to 14. While total OS levels slightly decreased from milking 1 to 14, relative levels of at least 10 sulfated OS increased over 3-fold (10 vs. 35%; $P < 0.03$) and were inversely correlated with sialic acid-containing milk OS. Future research should characterize how the observed marked changes in OS biosynthetic pathways during the early postpartum period may influence the newborn calf microbiome and immune system.

Key Words: oligosaccharides, sialic acid, capillary electrophoresis

81 Effect of acetate absorption on the daily rhythm of milk synthesis and plasma hormones and metabolites in dairy cows. C. Matamoros*[1], I. Salfer[2], and K. Harvatine[1], [1]*Department of Animal Science, The Pennsylvania State University*, *University Park, PA*, [2]*Dairy and Food Science Department, South Dakota State University*, *Brookings, SD*.

Time of feeding and nutrient absorption alters the daily rhythm of milk synthesis, likely by entrainment of the circadian clock of the mammary gland. Acetate supplementation increases milk fat in lactating cows and acetate also entrains circadian clocks in murine and cell models. The objective was to investigate the effect of time of acetate absorption on the daily rhythm of milk synthesis and plasma hormones and metabolites in dairy cows. Ten ruminally cannulated Holstein cows (127 ± 25 DIM; ± SD) were arranged in a 3 × 3 Latin square. Treatments were ruminal infusion of 10 mol/d of acetate as sodium acetate either over 22 h/d (CON) or over 8 h/d from 0900 to 1700 h (AM) or 2100 to 0500 h (PM). Experimental periods were 14 d with a 7-d washout period in between. Cows were milked every 6 h during the last 7 d of each period to assess the daily pattern of milk synthesis. Blood samples were taken 6 times each period to represent every 4 h over the day and plasma glucose, insulin, BHB, BUN, nonesterified fatty acids, and acetate were measured. Data were analyzed by cosinor-based rhythmometry to test the fit of a cosine function with a period of 24 h and calculate the acrophase (time at peak) and amplitude (peak to mean) of the rhythm. A 12 h harmonic model was also tested for plasma metabolites and hormones. Notably, AM increased the amplitude of milk yield by 70% and advanced the phase by 2 h and PM only advanced the phase by 1 h ($P < 0.05$ for all) and did not change the amplitude when compared with CON. Milk 16 carbon fatty acids fit a 24 h rhythm for AM and CON only, where AM increased the amplitude by 118% and delayed the phase by 1.6 h when compared with CON ($P < 0.05$). Plasma acetate fit a 24 h rhythm for all treatments. When compared with CON, AM increased the amplitude of plasma acetate by 129%, PM decreased the amplitude by 28%, and both treatments result in a phase shift ($P < 0.05$ for all). The rhythm of other variables of secondary interest were also affected by treatment. In conclusion, the results support a role of the timing of acetate absorption in entraining the daily rhythm of milk synthesis.

Key Words: short-chain fatty acids, nutrient entrainment, peripheral clocks

Production, Management, and the Environment

82 Cow longevity and culling on China dairy farms from 2013 to 2015. S. Liu*, J. Ma, and Z. Cao, *China agricultural university*, *Beijing, China*.

Few data are available on the descriptive characteristics of cow longevity and culling within a national population in China. The objective of this study was to describe cow longevity and the reasons associated with culling on dairy farms with more than 100 cows across China. In 2016, a nationwide survey was conducted, and 100 dairy farms in 12 provinces were involved. Culling records and the related data were obtained from management software and report system. Data were analyzed using SAS (version 9.0, SAS Institute Inc., Cary, NC, USA). Culling refers to the exit of cows from farms, as a result of death but regardless of reason, and cow-culling rate was calculated as the number of mature cow exits each year divided by the mean of mature cows stocked in the same year. The results showed that from 2013 to 2015, average culling rate were 24.7%, 22.9% and 30.4%, and average lactation age were 2.5, 2.7 and 2.9, respectively. 72% of culls were from the first 3 lactations, and the highest proportion of culls occurred in second lactation, accounting for 26.2%. Days in milk had effect on culling rate ($P < 0.01$), and most culling occurred before 60 d in milk, accounting for 27%. The causes of culling were also different ($P < 0.05$) with reproduction related reasons accounting for 20.9%, followed by digestive related reasons (20.7%), udder related reasons (13.7%), hoof related reasons (8.5%) and unknown reasons (8.4%). Voluntary culling accounted for 11.4%. In addition, the lactation age was affected by causes of culling ($P < 0.01$). Cows with reproductive diseases and respiratory diseases usually were culled in first 3 lactations, while cows with digestive system disorders, metabolic diseases, hoof diseases and udder diseases were often culled in higher lactation age (4–6 lactation). Culling rate was also related to farm scale($P < 0.01$). Dairy farms with 100 to 500 cows had the highest culling rate (32.9%), followed by dairy farms with 500 to 1000 cows (30.7%), dairy farms with 1000 to 2000 cows (27.5%), and dairy farms with more than 2000 cows had the lowest culling rate compared with other sale farms (23.4%, $P < 0.05$). This study provides an overview on dairy cow longevity and culling in China.

Key Words: mature cow, cow longevity, culling rate

83 Use of bulk tank milk fatty acid profiles as an on-farm decision-support tool. D. Warner, R. Lacroix, R. K. Moore, D. M. Lefebvre, and D. E. Santschi*, *Lactanet*, *Sainte-Anne-de-Bellevue, QC, Canada*.

Milk fatty acids (FA) originate from synthesis in the mammary gland (de novo), feed intake or body fat mobilization (preformed) or both origins (mixed). Rapid analysis of milk FA profiles through mid-infrared (MIR) spectroscopy could provide a valuable tool for feeding and management. Bulk tank samples from 3,395 dairy herds using milk recording in Quebec (CAN) are routinely tested by MIR. Samples (n = 573,000) between April 2019 and Feb 2020 were used to evaluate the use of FA as a monitoring tool. On a milk basis, each 0.1-unit increase in de novo FA was associated with an increase in fat by 0.201 units (R^2 0.67) and true protein by 0.117 units (R^2 0.65) whereas each 0.1-unit increase in preformed FA increased milk fat by 0.099 units (R^2 0.23) but had no association with true protein (R^2 0.01). Seasonal cycles showed decreased de novo and increased preformed FA during the summer months (FA basis). JE herds had a lesser drop in de novo FA than HO herds (−0.7 vs −1.3 g/100 g FA, respectively, for August vs April). Across seasons, JE herds had higher de novo (+1.5 g/100 g FA) but lower preformed FA (−2.9 g/100 g FA) and an overall greater milk fat content (+0.8%-units) as compared with HO herds. A snapshot analysis was conducted with herd averages for April 2019 for 2035 HO herds having high de novo (>median; HDN; mean ± SD of 27.9 ± 0.73 g/100 g FA) and low de novo (<median; LDN; 25.9 ± 1.14 g/100 g FA) levels. A mixed model approach was used in R with Gaussian distribution for continuous variables, and with binomial distribution and log-link function for variables with binary outcome, considering herd as a random effect. No differences existed in herd size, days in milk, and conventional vs organic, but HDN herds had higher odds (2.0; $P \leq 0.01$) of having a positive Transition Cow Index and lower odds (0.80; $P = 0.02$) of having a somatic cell count of 200 or more on test day. HDN herds had a 0.89 kg greater ($P \leq 0.01$) milk yield per cow on test day. A graphical and interactive tool was developed to visualize milk FA profiles with respective benchmarks and farm-specific historical data, was tested on farm and deployed in production via an IBM Cognos platform.

Key Words: fatty acid, Fourier-transform infrared, decision-support

84 Impacts of BiOWiSH application on greenhouse gases and air quality from liquid dairy manure. C. B. Peterson*, Y. Zhao, Y. Pan, and F. M. Mitloehner, *University of California, Davis*, *Davis, CA*.

With increasing focus on the environmental impacts of greenhouse gases (GHG) from livestock, decreasing the impacts of dairy production is of utmost importance. Dairy cattle waste can be a source of GHG emissions and criteria pollutants as well as a possible groundwater contaminant. Several manure additives have been studied as an approach to mitigate environmental impacts of liquid dairy manure (lagoon water). However, these additives have shown mixed efficacy. The present research aimed to test the commercial additive “BiOWiSH” on alterations of carbon dioxide, methane (CH_4), and nitrous oxide as well as ammonia. Lagoon water was collected from a commercial dairy in Solano County, CA and contained 0.34% solids, 99.7% moisture, and 0.026% total nitrogen with a pH of 7.68. Lagoon water was randomly allocated into treatment barrels (190 L each) with 6 replicates per treatment for the 3 treatments (n = 6; control, BiOWiSH Manure, and BiOWiSH Odor). Treatments were rehydrated according to product specifications (stock solution: 1 kg of treatment per 1,000 mL of DiH2O) and aliquots of 14 mL stock solution were applied every 9 d for 27 d (shock dose) followed by aliquots of 7 mL of stock solution applied every 9 d over an additional 27 d (maintenance dose), to the respective treatment barrels. Gaseous emissions were collected using flux chambers covering each barrel at designated intervals and measured in real time over a 54-d period. Chambers were connected to a mobile air quality emissions laboratory equipped with gaseous analyzers. The effects of treatments were compared against control. Results showed that BiOWiSH Manure versus the control increased emissions of CH_4 by 35.9%. BiOWiSH odor versus control also increased emissions of CH_4 by 85.1%. This additive may be applied as an alternate manure management strategy to increase CH_4 from covered dairy waste systems where CH_4 may then be trapped for subsequent use as a biogas. Further studies are needed to investigate the large scale repeatability of using this product as well as the mechanism of the BiOWiSH lagoon additives.

Key Words: sustainability, dairy cattle, lagoon additive

85 Evaluation of the GreenFeed method relative to the United States Pharmacopeia methodology validation guidelines. B. Garcia[1], S. Zimmerman*[1], and K. Schaefer[2], [1]*C-Lock Inc*, *Rapid City, SD*, [2]*DSM Nutritional Products*, *Basel, Switzerland*.

The GreenFeed (GF) gas flux (mass flowrate) measurement system is a non-invasive means of measuring enteric methane (CH_4) from ruminant animals. GF has been used for several purposes, including evaluating the effectiveness of feed additives [KM1] in reducing enteric CH_4 emissions. This study evaluated GF parameters using the United States Pharmacopeia < 1033 > §2.4 guidelines (USP) for instrument methodologies. Per USP guidelines, concentration measurements were evaluated using 5 certified CH_4 concentration mixtures (103.7–2104 ppm) in 3 replicates passed through the GF system. In addition, CH_4 flux measurements were evaluated by releasing gravimetrically measured ‘pulses’ of CH_4 at 5 different fluxes (70.7–509.7 g/d), with at least 3 replicates each, at a range of temperatures (4–29°C) and pressures (912–927 mbar) into the GF intake manifold. The tests were repeated with 2 unique GF machines to measure between unit variability. The USP requires repeatability and precision analysis using 'Intermediate Precision' (IP) reported as %Geometric Coefficient of Variation, using mixed linear models (MLM). Significance of random effects was determined by ratio tests between a model with a given random effect and a model without. Singular (zero) variance estimates were removed. The MLM were computed using the 'lmer' function of the 'lme4' library for the R platform, while ratio tests used the 'anova' function. CH_4 flux estimates had an IP of 6%, while concentration estimates had an IP of 2%. The GF measurements were found to be highly correlated to certified concentration standards and gravimetric fluxes (0.9993, 0.9972). The GF system estimated CH_4 fluxes with 1.92% average relative bias. Concentration estimates had 0.15% average relative bias. Concentration variance estimates were non-singular for the pressure of the environment, while flux variance estimates were non-singular for pressure and replicate. Temperature, and the GF unit performing the measurement were singular effects. In summary, GF produces repeatable and accurate emissions measurements compared with gravimetric measurements or certified concentration standards.

Key Words: methane, measurement, emissions

86 An environmental assessment of dairy farms in the eastern United States.

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There is need for a comprehensive assessment of the environmental impacts of dairy farms at regional and national scales to better track improvements made by the industry. A methodology using process-level simulation and cradle to farm-gate life cycle assessment has been applied to the eastern United States with plans for completing all regions of the country. Representative dairy farms of various sizes and management practices are simulated with the Integrated Farm System Model using the soil characteristics and climate where farms are located. Farm-gate footprints are determined by totaling values among farms and locations within the region considering the amounts of milk produced by each. Northeastern dairy farms were determined to emit 12,455 ± 1,100 Gg CO_2e of greenhouse gas with an intensity of 0.99 ± 0.09 kg CO_2e per kg of fat and protein corrected milk (FPCM) produced. Fossil energy consumption was 33,542 ± 5,300 TJ or 2.68 ± 0.42 MJ per kg FPCM. Blue (non-precipitation) water consumption was 193 ± 42 Tg with an intensity of 15.4 ± 3.4 kg per kg FPCM. A total of all forms of reactive N loss was 108 ± 13 Gg with an intensity of 8.6 ± 1.0 g per kg FPCM. These metrics were equivalent to 1.5% of the greenhouse gas emissions, 0.32% of fossil energy use and 0.87% of fresh water consumption reported by governmental agencies for recent years covering all states in the region. Thus, greenhouse gas emissions, fossil energy use and blue water use associated with dairy farm production in this region are relatively small compared with total estimates. Simulated emissions of volatile organic compounds were also within 2% of governmental estimates for the region. The greatest environmental concern appears to be that of ammonia emission, where dairy farms accounted for 65% of governmental estimates for the region. Environmental footprints were found to vary widely among farms as influenced primarily by soil characteristics and climate and secondarily by farm management. Therefore, prescriptive mitigation strategies for individual farms is more effective than uniform enforcement of specific strategies.

Key Words: life cycle assessment, greenhouse gas, nitrogen loss

87 National consumer survey of dairy food preferences and purchase interest.

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The United States' dairy industry faces increasing competition from plant-based dairy mimetics, fluctuating export markets, and animal welfare criticism. Small and medium-sized dairy farms are vanishing. Improved understanding of American consumers' attitudes and desires could enable small dairy processors to expand markets and sustain smaller farms through niche product marketing. We conducted an Internet survey in the summer of 2019. At least 400 adults aged 18 years or older were recruited from each US state by Dynata (n = 20,040). National, regional, and state results were tabulated. Data were analyzed by chi-squared and ANOVA, with a significance level of $P = 0.05$. Nearly 97% of the respondents consume dairy foods. Fewer than 50% of respondents were aware of local dairy farms, processing, or local products sold in retail stores. Residents of the New England, East North Central, and Mid-Atlantic regions were more knowledgeable of local dairy businesses and products. The respondents who consume dairy foods were aware of the farm locations only 25% of the time spent shopping; the consumers knew where the products were processed 24% of the time, and they read the product labels only 31% of the time to find that information. When asked how label claims would influence their willingness to pay (WTP) for conventional dairy products from larger, regional, and national processors, survey participants said they would pay less for raw products, and more for local products. Cows' milk was preferred by 92%, and 72% preferred cheese made from cows' milk. Vermonters and people aged 55 or older rated the visual, taste, and nutritional quality of milk, cream, butter, yogurt, cheese, and frozen desserts higher than the national mean scores on a 7-point scale. The most-preferred package size (61% of respondents) for milk was one gallon, but only 49% of Mid-Atlantic residents chose that option. One-third of respondents chose clear plastic containers as best for milk. The data from this survey guided the development of an online visualization tool for farmers and processors to better understand dairy food preferences among consumers in their marketing area.

Key Words: consumers, local, purchasing

Ruminant Nutrition: General

88 Effect of 25-hydroxyvitamin D$_3$ [25-(OH)D$_3$] on dairy cows. H. M. Golder* and I. J. Lean, *Scibus*, *Camden, NSW, Australia*.

Subclinical hypocalcemia prevalence remains high postpartum despite use of negative DCAD diets and vitamin D$_3$. We hypothesized that feeding 25-(OH)D$_3$ (25D) during lactation, and in transition in conjunction with negative DCAD diets, would improve milk production, reproduction, and health. Dairy cows were used in 2 randomized exposure experiments. Experiment 1; cows in Control [CON; n = 645; no 25D] or Treatment [TRT; n = 537; 2 mg/d of 25D prepartum and 1 in lactation] groups assigned and fed from ~21 d prepartum were monitored for milk yield and composition, and health and reproductive measures. Experiment 2; 4 groups of cows (median 147 DIM) were monitored per Experiment 1 to the end of that lactation (L1), subsequent transition and lactation (L2). Groups were (1) CON-CON (no 25D), (2) TRT-TRT (1 mg/d of 25D in L1 and L2 and 2 prepartum), (3) CON-TRT (1 mg/d of 25D in L2 and 2 prepartum), and (4) TRT-CON (1 mg/d of 25D in L1). For L1, 1,032 cows entered control groups 1 or 3 and 1,032 in groups 2 or 4. The *n*/group that entered L2 was 521, 523, 273, and 248, respectively. Analysis used mixed linear and survival models (STATA V15, Statcorp LP, College Station, TX). Blood Ca, P, and 25D concentrations (n = 17/group) were evaluated at 5 times. Experiment 1, TRT cows had 0.2 lower LN SCC than CON cows ($P = 0.002$) and multiparous (multi) TRT cows had 41.1 ± 23.4% (95% CI = 2.0–95.3%) higher odds of pregnancy/d than multi CON cows, reducing days open by a median of 22 d. Primiparous TRT cows had 1.67 ± 0.40 times greater odds of mastitis/d than primiparous CON cows. In Experiment 2, TRT-TRT cows had 15.5–28.9% lesser odds to be bred/d than other groups ($P = 0.016$). Multi CON-CON and TRT-CON cows had 21.4 ± 7.8% and 30.3 ± 16.6% greater odds of pregnancy, respectively, than multi TRT-TRT cows. Serum Ca concentrations were not affected by group ($P = 0.988$), P ($P = 0.003$) and 25D concentrations ($P < 0.001$) were highest in the TRT-TRT cows. Duration on transition diet improved many measures of production, health and reproduction. While treatment lowered SCC and provided other benefits, particularly for multi cows including the time to pregnancy, responses varied across treatment groups.

Key Words: calcidiol, calcium, prepartum

89 Bioefficacy of hydroxy-selenomethionine in dairy cows. N. L. Whitehouse*[1], J. R. Sexton[1], S. M. Hollister[1], L. H. P. Silva[2], S. M. Fredin[3], and M. Briens[1], [1]*University of New Hampshire*, *Durham, NH*, [2]*Adisseo USA Inc*, *Alpharetta, GA*, [3]*Adisseo France SAS*, *Commentry, France*.

Selenium (Se) is an essential trace mineral that if deficient in the soil will therefore be deficient in diets fed to dairy cows. Selenium supplements exists in inorganic and organic forms, with the organic form being seleno-yeasts (SY) or pure forms such as selenomethionine or hydroxy-selenomethionine (OH-SeMet). The objective was to determine the amount of Se that was transferred to milk and blood of mid to late lactation dairy cows when supplemental Se from a OH-SeMet (Selisseo 2% Se, Adisseo France SAS) was fed compared with an unsupplemented group and a group supplemented with a SY. Twenty-four lactating Holstein cows, 12 multiparous and 12 primiparous (178 ± 43 d in milk (DIM)) were used in a randomized complete block design for 91 d (7 d for covariate and 84 d for treatments). Treatments were 1) basal (practical) diet with an expected Se background of 0.2 mg Se per kg as-fed (negative control), 2) basal diet + 0.3 mg Se per kg as-fed from SY (positive control SY-0.3), 3) basal diet + 0.1 mg Se per kg as-fed from OH-SeMet (OH-SeMet-0.1), and 4) basal diet + 0.3 mg Se per kg as-fed from OH-SeMet (OH-SeMet-0.3). Blood (at 1030h) and milk samples (am and pm milking) were obtained from each on the last 3 d of the covariate week and wk 1, 2, 3, 4, 6, 8, 10 and 12 of the study. Data were analyzed using the PROC MIXED procedures of SAS with REPEATED measures. Significance was declared at $P \leq 0.05$. Plasma and milk Se concentrations was highest for OH-SeMet-0.3 (Table 1). However, there was no difference on the plasma glutathione peroxidase activity between groups. Those results are a confirmation that organic Se forms can increase milk and plasma Se concentrations. Moreover, when administered at the same level of supplementation, OH-SeMet showed to be more efficient than SY to improve those Se concentrations.

Key Words: selenium, selenomethionine, milk

90 Effect of electrochemically activated drinking water on production performance and somatic cell counts in dairy cows. E. Vargas-Bello-Pérez*, S. Cruz-Morales, R. Dhakal, and H. H. Hansen, *Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen*, *Frederiksberg C, Denmark*.

Electrochemically activated water (ECW) can be used for the prevention and control of microorganism, however, there is scarce information about its use in dairy farms. Thus, the objective of this study was to determine the effect of ECW on production performance and somatic cell counts in dairy cows. For 6 mo, 2 groups of lactating Red Danish cows were fed regular drinking water (n = 27) and ECW (n = 27) water dosed with 4 ppm of 29 mg/L of chlorate (Neuthox, Danish Clean Water, Denmark). Monthly records from milk production, milk composition and somatic cell counts were analyzed. Milk production, composition and SCC data were analyzed with linear mixed models using R version (3.5.1). Bacteriological conditions (BC) at the beginning of the study for non-dosed and dosed drinking troughs were >200 MPN/100 mL of coliform bacteria at 37°C, 120 MPN/100 mL of *Escherichia coli*, >3000 cfu/mL of bacterial count at 22°C and 1100 cfu/mL of bacterial count at 37°C. Then, after 6 mo, BC in dosed drinking troughs were <1 vs. >200 MPN/100 mL of coliform bacteria at 37°C, <1 vs. 200 MPN/100 mL of *Escherichia coli*, 160 vs. >3000 cfu/mL of bacterial count at 22°C and 150 vs. >3000 cfu/mL of bacterial count at 37°C. Milk yield (32.4 ± 3.2 kg/cow), milk fat (4.55 ± 0.38%) and milk protein (3.78 ± 0.16%) were similar ($P > 0.05$). Somatic cell counts ($\times 10^3$/mL) were significantly ($P < 0.05$) lowered by ECW (162 ± 42) compared with non-dosed animals (411 ± 202). Overall, results showed that ECW could be an alternative to reduce somatic cell counts without detrimental effects on milk production and milk composition.

Key Words: milk production, electrochemical water, somatic cells

Table 1 (Abstr. 89). Average plasma and milk Se concentration and plasma glutathione peroxidase activity

Item	Negative control	Positive control SY-0.3	OH-SeMet-0.1	OH-SeMet-0.3	*P*-value
Plasma Se, ng/mL	120^{d}	134^{b}	122^{c}	142^{a}	<0.0001
Milk Se, ng/mL	57^{d}	97^{b}	78^{c}	116^{a}	<0.0001
Glutathione peroxidase, nmol/mL	90.58	91.63	91.00	92.85	0.859

$^{a–d}$Means within rows differ at $P < 0.05$.

91 Inclusion of calcium-magnesium tetrahydroxide as a dietary source of inorganic Mg alters ruminal pH and fermentation in a dual-flow continuous culture system. J. A. Arce-Cordero*, H. F. Monteiro, V. L. N. Brandao, X. Dai, S. Bennett, J. Vinyard, and A. P. Faciola, *Department of Animal Sciences, University of Florida*, *Gainesville, FL.*

Ruminal acidosis is a major issue affecting modern cattle industry. Buffers such as sodium sesquicarbonate are normally fed to reduce negative effects of acidosis; however, some mineral sources may play a role as controllers of ruminal pH. We aimed to evaluate the effects of inclusion of $CaMgCO_3$ and $CaMg(OH)_4$ on microbial fermentation, hypothesizing that their inclusion as supplemental sources of inorganic Mg (iMg) would allow for similar ruminal fermentation conditions to those observed in a positive control diet formulated with MgO as the sole source of iMg plus a buffer. Four treatments were defined by the main source of iMg in the diet: (1) CO_3 [100% iMg from $CaMg(CO_3)_2$]; (2) CO_3/OH [50% iMg from $CaMg(CO_3)_2$, 50% iMg from $CaMg(OH)_4$]; (3) OH [100% iMg from $CaMg(OH)_4$]; (4) MgO/Na (100% iMg from MgO, plus 0.6% sodium sesquicarbonate). Nutrient concentration was the same across treatments (16% CP, 30% NDF, 1.69 MCal NEl/kg, 0.68% Ca, and 0.22% Mg from which 0.05% corresponds to iMg). Four fermenters were used in a 4 × 4 Latin square design with 4 periods of 10 d each. In the last 3 d of each period samples were collected for analyses of nutrient digestibility, soluble Mg, VFA, and NH_3-N, while pH was measured at 0, 1, 2, 4, 6, 8, and 10 h post morning feeding to estimate hours below pH of 6 (pH < 6) and area under the pH curve (pH AUC). Bacteria pellets were harvested for ^{15}N analysis and estimates of N metabolism. Treatment effects were analyzed with PROC MIXED of SAS, while effects of $CaMgCO_3$ and $CaMg(OH)_4$ were evaluated by orthogonal contrasts. Inclusion of $CaMg(OH)_4$ increased pH AUC ($P = 0.02$); molar proportion of butyrate ($P = 0.02$), and decreased pH < 6 ($P < 0.01$), showing a similar effect to that one observed in the positive control treatment formulated with MgO and buffer. No effects of $CaMgCO_3$ and $CaMg(OH)_4$ were found on soluble Mg, nutrient digestibility and N metabolism. Results indicate that feeding $CaMg(OH)_4$ as a mineral source may aid in the control of ruminal pH.

Key Words: in vitro, acidosis, buffers

92 Effects of reduced dietary cation and anion difference on production, nutrient digestibility, and urine pH in lactating cows. C. Lee*[1], J. E. Copelin[1], L. R. Rebelo[1], P. A. Dieter[1], W. P. Weiss[1], and T. Brown[2], [1]*Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University*, *Wooster, OH*, [2]*Dairy Nutrition Plus*, *Ralston, IA.*

Manure acidification is an effective strategy to lower ammonia emissions from manure and increase its value as fertilizer. Lowering dietary cation and anion difference (DCAD) can reduce urine pH and this may acidify manure depending on the degree of pH reduction in dairy cows. However, reducing DCAD can negatively affect DMI, production and fiber digestibility. The objective was to examine lactating cows fed a diet with reduced DCAD on production, fiber digestibility, and urine pH. Twenty cows were used in a randomized block design and fed 1 of the following 3 diets: a diet with DCAD of 220, 150, or 75 mEq/kg (n = 6, 7, and 7, respectively; DCAD, Na + K − Cl − S; 16.8% CP, 33% NDF on DM basis). SoyChlor replacing soybean meal and corn grain was used to decrease DCAD. The experiment was conducted for 5 wk to monitor DMI and production and spot fecal samples were collected in wk 5 to estimate fecal output and apparent total-tract nutrient digestibility. In wk 5, urine samples were also collected (every 4 h in a 24-h cycle) to estimate urine volume (creatinine) and measure urine pH. Data were analyzed using the MIXED procedure of SAS (block as random and diet as fixed effect). Reducing DCAD lowered (25.3 to 23.6 kg/d; $P = 0.02$) DMI in a linear manner. Milk yield, ECM, and ECM/DMI were not different ($P > 0.26$) among treatments. Milk component contents and yields were not affected by level of DCAD. Total-tract apparent digestibility of DM and NDF did not differ among treatments. Estimated urine volume increased (36 to 44 kg/d; $P < 0.01$) and urine pH decreased (8.25 to 7.86; $P < 0.01$) in a linear manner with decreasing DCAD. In conclusion, lowering DCAD of a lactation diet from 220 to 75 mEq/kg decreased DMI and numerically decreased milk yield (38.4 to 36.9 kg/d). However, the decreases in DCAD did not affect DM and NDF total-tract digestibility. Although an increase in urine volume and decrease in urine pH with decreasing DCAD was observed, we do not know whether these changes are effective in lowering ammonia emission from manure.

Key Words: dietary cation and anion difference, digestibility, and urine pH

93 Effects of dietary antioxidants and modulators of immune response on animal performance and metabolism of Holstein cows during heat stress. A. Ruiz Gonzalez*[1,2], W. Suissi[2], L. H. Baumgard[3], P.-Y. Chouinard[1], R. Gervais[1], and D. E. Rico[2], [1]*Université Laval*, *Quebec, QC, Canada*, [2]*CRSAD*, *Deschambault, QC, Canada*, [3]*Iowa State University*, *Ames, IA.*

Twelve multiparous Holstein cows (42.2 ± 5.6 kg milk/d; 83.4 ± 27.1 DIM) were used in a split-plot design testing the effects of mineral and vitamin supplementation on animal performance and metabolism during heat stress. The main plot was the level of dietary vitamin E and Se (HVE: 200 IU/kg and 1.2 ppm; LVE 20 IU/kg and 0.3 ppm; respectively). Within each plot, cows were randomly assigned to 1) Heat stress (HS), 2) Pair-feeding (PF), or 3) HS with Vitamin D and Ca supplementation (HS+DCa; 1820 IU/kg and 1.5% Ca) in a Latin square design with 14-d periods. Milk components were analyzed by mid-infrared spectroscopy from samples taken on d 0, 3, 7, 10, and 14. This statistical model included the random effects of cow and period, and the fixed effects of plot, treatment, day and their interactions. No 3-way interactions were detected for any variable. Heat stress progressively decreased dry matter intake (DMI) before stabilizing on d 7 (30% reduction; Time $P < 0.001$) and was not affected by treatment. Milk yield decreased progressively in all treatments and was higher in PF relative to both HS and HS+DCa cows from d 3 to 14 (−10%; $P < 0.05$). There was a treatment by day interaction for milk fat and protein concentrations ($P < 0.05$). Milk fat was 10% lower in HS relative to PF on d 10 and 14, but not different between PF and HS+DCa, whereas milk protein was 7.5% lower in HS and HS+DCa relative to PF from d 3 to 14. Pre-prandial NEFA were 64% lower, whereas pre-prandial insulin was 58% higher in HS and HS+DCa than in the PF on d 7 and 14 ($P < 0.05$). Mineral and vitamin supplementation seems to have a moderate effect on the performance and metabolic responses of cows to heat stress.

Key Words: heat stress, dairy cows, nutrition

Small Ruminant

94 Productive performance, milk composition, and milk fatty acids of goats supplemented with sunflower and linseed whole seeds. E. Vargas-Bello-Pérez*[1], C. A. García Montes de Oca[2], N. Pescador-Salas[2], J. G. Estrada Flores[3], J. Romero Bernal[2], L. E. Robles Jimenez[2], and M. González-Ronquillo[3], [1]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen*, *Frederiksberg C, Denmark*, [2]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México*, *Toluca, Estado de México, México*, [3]*Instituto en Ciencias Agropecuarias y Rurales, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México*, *Toluca, Estado de México, México*.

The objective of this study was to determine productive performance, milk composition and milk fatty acids (FA) of goats supplemented with sunflower and linseed whole seeds in grass silage-based diets. Nine Alpine goats were grouped in a replicated 3 × 3 Latin square design (n = 3) that included 3 21 d periods. Treatments were based on grass silage offered ad libitum and a concentrate mixture supplemented with either 40 g/d of Megalac (control), 80 g/d of sunflower seed (SF), or 80 g/d of linseed (LS). Data was analyzed using GLM procedure of SAS. Milk yield (776 ± 20 g/d), dry matter intake (64 ± 3 g/kg $LW^{0.75}$) and digestibilities (g/kg) of dry matter (612 ± 27), organic matter (664 ± 12), neutral detergent fiber (567 ± 24) and acid detergent fiber (522 ± 28) were not affected by treatments ($P > 0.05$). Treatment did not affect milk fat yield (40 ± 1.1 g/d), protein content (4.5 ± 0.04%) and protein yield (35 ± 1.2 g/d). Milk fat content was higher ($P < 0.05$) in control (5.4 ± 0.1%), intermediate in SF (5.1 ± 0.1%) and lower (4.9 ± 0.1%) in LS. Compared with control, SF and LS, decreased C16:0 (28.2 ± 0.8 vs. 23.1 ± 0.8 and 22.4 ± 0.8 g/100g) and increased total C18:1 (24.1 ± 1.1 vs. 27.6 ± 1.1 and 28.4 ± 1.1 g/100g) respectively. The ratio of monounsaturated to polyunsaturated FA was higher ($P < 0.05$) in LS (8.9 ± 0.32), intermediate in SF (7.7 ± 0.32) and lower in control (6.2 ± 0.32). Overall, SF and LS represent an effective strategy for altering the FA composition of goat's milk toward a healthier profile for humans without deleterious effects on animal performance.

Key Words: oilseeds, milk, dairy goat

95 Physiological, lactational, and blood metabolite profile of dairy goats exposed to low ambient temperatures. W. Coloma-Garcia[1,2], N. Mehaba[1], X. Such[1], G. Caja[1], and A. A. K. Salama*[1], [1]*Research Group in Ruminants (G2R), Universitat Autonoma de Barcelona*, *Barcelona, Spain*, [2]*Facultad de Medicina Veterinaria, Universidad Agraria del Ecuador*, *Guayaquil, Ecuador*.

Low winter temperatures in some regions combined with increasingly frequent extreme cold waves have a negative impact on animal performance, behavior, and welfare. The objective of this study was to evaluate the physiological, metabolic, and lactational responses of dairy goats to low ambient temperatures during 3 weeks. Eight Murciano-Granadina dairy goats in mid-lactation were divided into 2 balanced groups and randomly assigned to 2 treatments: thermoneutral (TN; 15 to 20°C) and low temperature (LT; −3 to 6°C). The experimental design was a crossover with 2 treatments in 2 periods (21 d each). Goats received a total mixed ration (70% forage and 30% concentrate) and water ad libitum and were machine-milked twice daily (8 and 17 h). Feed intake, water consumption, rectal temperature, and respiration rate were recorded daily. Milk samples for composition were collected weekly. Insulin, glucose, nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), cholesterol, and triglycerides were measured in blood. Body weight was recorded at the start and end of each period. Compared with TN goats, LT goats had similar feed intake and milk yield, but lower ($P < 0.05$) water consumption (−23%), respiratory rate (−6 breaths/min) and rectal temperature (−0.32°C). Furthermore, milk of LT goats had greater ($P < 0.05$) contents of fat (+12%), protein (+11%), and lactose (+4%). The LT goats lost −0.45 kg BW, whereas TN goats gained 2.2 kg (SE of the difference = 0.77; $P < 0.05$). Insulin and cholesterol blood levels were not affected by LT. However, values of blood glucose, NEFA, hematocrit and hemoglobin were increased ($P < 0.05$) by LT, whereas BHBA and triglycerides decreased ($P < 0.05$). In conclusion, Murciano-Granadina dairy goats during lactation were sensitive to cold temperatures. The LT goats maintained feed intake and milk production, but produced milk with greater fat and protein contents. The LT goats mobilized body fat reserves to cover the extra energy needed for heat production under cold conditions.

Key Words: cold temperature, milk production, metabolism

Dairy Foods: Processing

96 Effect of heat treatment conditions and pH on physicochemical properties and protein denaturation of liquid milk protein concentrate. N. Rafiee Tari*[1], A. Guri[2], Z. Gaygadzhiev[3], and A. Wright[1], [1]*Department of Human Health and Nutritional Sciences, University of Guelph*, *Guelph, ON, Canada*, [2]*Gay Lea Co-operative Foods Ltd*, *Hamilton, ON, Canada*, [3]*Reckitt Benckiser*, *Evansville, IN*.

Milk protein concentrates (MPC) are high-protein ingredients with unique functional and nutritional properties that can be tailored through modifying processing conditions, including temperature, pH, filtration conditions, and drying. MPC is typically dried to produce powdered ingredients. However, the effect of the processing conditions on structural and functional properties of liquid MPC remains to be thoroughly understood. In this study, the pH of liquid MPC (13% protein and 18.5% total solids) was adjusted to either 6.5, 6,7 or 6.9, followed by heat treatment at 85°C/5min or 125°C/15s. The extent of whey protein denaturation was analyzed by HPLC. Heat treatment at all studied pHs resulted in substantial denaturation of whey proteins, with β-lactoglobulin denatured more extensively than α-lactalbumin. More extensive whey protein denaturation was also observed for MPC heated at 125°C/15s compared with 85°C/5 min, irrespective of pH. Changes in liquid MPC structural properties were monitored over time; 1, 4 and 8 d after the process. Viscosity increased over time, regardless of temperature/time and pH, suggesting the role of whey proteins denaturation and aggregation, and their interaction with casein micelles. MPC at pH 6.9 had a significantly higher viscosity than MPC at pH 6.5 or 6.7, at both temperature/time conditions. Mean particle size of the MPC samples did not change significantly over time. However, the particle size distribution showed the formation of larger particles after 4 and 8 d of cold storage in samples heat treated at pH 6.9. This study showed that the degree of whey protein denaturation and inter- and intramolecular interactions of milk proteins are affected by pH and heat treatment conditions of liquid MPC. The findings provide a better understanding of the processing factors contributing to physicochemical properties of liquid MPC, which can be employed to develop milk protein ingredients with tailored structural and functional properties.

Key Words: liquid MPC, heat treatment conditions, pH

97 Reconstitution properties of micellar casein powders with different calcium contents and their production. J. Schäfer[1], R. Kohlus[2], J. Hinrichs[1], and Z. Atamer*[1], [1]*Department of Soft Matter Science and Dairy Technology, Institute of Food Science and Biotechnology, University of Hohenheim*, *Stuttgart, Germany*, [2]*Department of Process Engineering and Food Powders, Institute of Food Science and Biotechnology, University of Hohenheim*, *Stuttgart, Germany*.

Micellar casein (mCN) powders have poor reconstitution properties, in particular at low temperatures. The solubility of milk protein powders can be improved by reducing calcium content. Therefore, the study aimed (1) to manufacture calcium reduced mCN powder on a technical scale, and (2) to investigate the reconstitution properties of mCN powders with different calcium contents. For the production of a high-calcium depleted mCN concentrates, the skim milk was first acidified to pH 6.2 (1), and microfiltered (0.1 μm) (2). The retentate was acidified to pH 5.6 (3), and microfiltered in diafiltration modus using demineralized water in 6 steps (4). Finally, the obtained retentate was spray dried (5). For comparison purposes, a slightly-calcium reduced mCN powder was also manufactured. As a reference, non-calcium-depleted mCN was manufactured via concentrating the skim milk using microfiltration and spray drying the resulting retentate. The influence of the calcium content on the solubility and the effect of drying on particle size, apparent viscosity, sol-gel transition, were investigated. A technical-scale production of mCN powder containing different levels of calcium was shown to be possible. The solubility experiments showed that the non-calcium reduced spray-dried powder (calcium content: 19820 ± 330 mg kg^{-1}) had 0.5 ± 0.1 mL sediment, whereas the calcium-reduced micellar casein powders (slightly depleted: calcium content: 27170 ± 10 mg kg^{-1} and highly depleted: calcium content: 15250 ± 210 mg kg^{-1}) contained 3.8 ± 1.1 mL and 3.4 ± 0.1 mL, respectively, indicating a significantly decreased solubility due to calcium depletion ($P < 0.05$). The particle size of the reconstituted high-calcium depleted mCN concentrate was significantly bigger (206.3 ± 8.8 nm) ($P < 0.05$) compared with non- and slightly-calcium depleted mCN concentrates (172.0 ± 2.1 and 174.8 ± 8.1 nm), which were not significantly different in their sizes.

Key Words: solubility, calcium depletion, casein concentrate

98 Effect of temperature on the performance of plate-and-frame filtration during milk protein concentrate manufacture. A. Mishra* and L. E. Metzger, *South Dakota State University*, *Brookings, SD*.

In the typical milk protein concentrate (MPC) manufacturing process skim milk is ultrafiltered using spiral wound membrane to produce MPC with 20% total solids (TS) and more than 80% total protein (TP) based on solids. This MPC is then further concentrated before spray drying using reverse osmosis or nanofiltration. In this study we had attempted to concentrate MPC using a plate-and-frame filtration (PF) system instead of spiral wound system. Three replicates of MPC80 having TS 20.01, 19.70 and 20.65% from the ultrafiltration of skim milk were concentrated in a PF system fitted with flat sheet membranes (Alfa Laval M37; surface area 3.3 m^2 with a 10 kDa molecular weight cut-off). Three different PF settings were utilized including: PF at 22°C (PF22); PF at 50°C for medium solids (PF50MS); PF at 50°C for high solids (PF50HS). Filtration was continued until the transmembrane pressure difference was 9 bar for PF22 and PF50HS. For the PF50MS, filtration was stopped when the TS achieved was 30%. The average flux, final TS and TP/TS ratio were 8.76, 10.50 and 11.18 L/m^2hr, 26.83, 29.92 and 34.24% and 0.83, 0.88 and 0.87 respectively for the PF22, PF50MS and PF50HS treatments. The average flux, final TS and TP/TS ratio were significantly ($P < 0.05$) higher for the treatments conducted at 50°C as compared with 22°C. The viscosity of the retentates corresponded to the final TS and was 577, 1513 and 12805 cP for the PF22, PF50MS and PF50HS treatments, respectively. The permeate from the PF50HS treatment had significantly ($P < 0.05$) higher solids and TP as compared with the other treatments. The standard plate count (SPC) of the retentate before drying was significantly ($P < 0.05$) higher for the treatments at 50°C and was 3.54 to 4.41, 5.17 and 5.28 log10cfu/g for the PF22, PF50MS and PF50HS treatments, respectively. The loose and tapped density of MPC powders after spray drying were significantly ($P < 0.05$) lower for the PF50MS and PF50HS treatments. This study determined that, increasing the PF temperature improves filtration performance and the level of TS achieved but also increase the SPC count of the final retentate.

Key Words: milk protein concentrate, plate-and-frame filtration, quality

99 Processing of high-protein yogurt from milk concentrates: Power ultrasound as an innovative tool to generate specific texture properties. A. Körzendörfer* and J. Hinrichs, *University of Hohenheim*, *Stuttgart, Germany*.

The fermentation of preconcentrated milk is a challenging method to avoid acid whey during the manufacture of high-protein fermented milks like Greek yogurt and skyr. Since acid whey is undesired as a by-product due to environmental concerns, alternative processes that involve a concentration step before the fermentation, e.g., by microfiltration, are of economic and ecological interest. However, acid milk gels from concentrated milk (>8% protein) exhibit a high interconnectivity and an excessive firmness, resulting in technical and sensory problems. As the reduction of the microgel particle size is impaired, products are often not smooth and perceived as too viscous. The aim of our research was to improve the processing of high-protein yogurt from concentrates. For this purpose, power ultrasound (US) was applied as an innovative post-processing technology. Skim milk was fortified with milk protein powder to 10% protein, heated, and fermented at 43°C. Fermentations were stopped at pH 5.0, 4.8, and 4.6, respectively, by breaking up the gel with a perforated disc and immersing the containers in iced water. Yogurts were processed into stirred yogurt by shearing. Half of the samples were additionally treated with an US sonotrode for 5 s at a frequency of 20 kHz. Several physical properties were then analyzed. The short-term sonication considerably decreased the apparent viscosity of the yogurts by 30–40%, whereas the particle size and water-holding capacity were only slightly affected. Further experiments were performed by studying the effects of sonication time. Strong negative exponential correlations ($R^2 > 0.99$) were found between the sonication time and various rheological properties (storage modulus, apparent viscosity, yield stress). Moreover, the visual smoothness of the yogurts increased with the sonication time. The application of power US is a highly effective tool to control the rheological properties of high-protein fermented milks from concentrates. This offers the potential to develop innovative products and will help to establish sustainable processes.

Key Words: Greek yogurt, ultrasound, fermented milk product

100 Freezing high-pressure-jet–treated ice cream mix: A study of the kinetics and microstructure. G. L. Voronin*, G. Ning, J. N. Coupland, R. Roberts, and F. M. Harte, *The Pennsylvania State University*, *University Park, PA*.

High-pressure-jet (HPJ) processing, a recent development in high pressure technology, has been shown to enhance foaming, emulsifying, and rheological properties in a variety of dairy systems including skim and whole milk. Recently, we showed a 400 MPa HPJ treatment of low fat ice cream mix resulted in an ice cream mix with an increased consistency coefficient and reduced apparent ice crystal growth compared with a non-HPJ-treated control due to the formation of fat-protein complexes at 400 MPa, which were visualized using confocal scanning laser microscopy (CSLM). These results suggest potential for eliminating the need for hydrocolloids and emulsifiers in an ice cream formulation, however these benefits have not been demonstrated in a dynamically frozen ice cream. The objectives of the current work were to characterize the physical properties (overrun, apparent viscosity, particle size) of HPJ-treated (100–500 MPa) low fat ice cream (6% fat) throughout dynamic freezing and to determine the melting rate, hardness, and microstructure of the final ice cream after hardening. At each pressure, triplicate low-fat ice cream batches were produced. All results were analyzed using one-way ANOVA to identify significance at a 0.05 level ($P < 0.05$) with Tukey's test applied for mean comparisons. A combination of CSLM and transmission electron microscopy revealed unique microstructural components in ice creams treated at HPJ pressures ≥400 MPa including coalesced milkfat coated with disrupted casein micelles. The ice creams treated at these pressures (≥400 MPa) also had an increased apparent viscosity (when melted, 26.2 mPa.s at 500 MPa), hardness (3,824 g at 500 MPa), and melting rate (2.6 g.min^{-1}at 500 MPa) compared with a non-HPJ-treated control (viscosity = 12.9 mPa.s, hardness = 2,506 g, and melting rate = 2.2 g.min^{-1}). These differences were attributed to the unique microstructure developed during HPJ treatment. By altering the microstructure, apparent viscosity, and hardness of a low fat ice cream, HPJ technology shows some promise for alleviating some common ice cream defects including ice cream shrinkage and iciness.

Key Words: high-pressure jet, ice cream, transmission electron microscopy

Production, Management, and the Environment

101 Management strategies impact milk fatty acid production and its relationship with milk composition. S. C. Allen*[1], D. M. Barbano[2], D. H. Poole[1], J. Odle[1], M. A. Drake[1], and S. H. Ward[1], [1]*North Carolina State University*, *Raleigh, NC*, [2]*Cornell University*, *Ithaca, NY*.

Ten herds (n = 6 Jersey, JE, n = 3 Holstein, HO, n = 1 mixed) were enrolled to determine the impact of management practices and breed on milk FA composition. Bulk tank samples were collected monthly (7 samples/mo) and analyzed for milk and FA composition (de novo, DNFA; mixed origin, MOFA; and preformed, PRFA). Data were analyzed using PROC CORR and REG of SAS. A positive relationship was observed between milk fat %, protein % and DNFA in JE herds (r = 0.7473 and 0.6474, $R^2 = 0.5571$ and 0.6474, $P < 0.0001$). HO herds showed similar results for fat and protein % (r = 0.7476 and 0.7433, $R^2 = 0.8653$, respectively, $P < 0.0001$). DNFA and milk protein (kg) were positively correlated in JE and HO herds (r = 0.8053; $R^2 = 0.6474$, JE; r = 0.8628, $R^2 = 0.7433$, HO; $P < 0.001$). A stronger relationship was observed between MOFA and milk protein % with HO herds than JE herds (r = 0.8341 and 0.4849, $R^2 = 0.2329$ and 0.6944 and 0.2329, respectively, $P < 0.001$). Herd visits were conducted quarterly to collect body condition and locomotion scores, stocking rates, and ration composition and particle size. Herds were classified as high and low de novo (HDN, LDN), mixed origin (HMO, LMO), and preformed (HPF, LPF) at each visit. Data were analyzed using PROC GLIMMIX of SAS; herd classification was the fixed effect. Means were separated using Fisher's LSD, and significance was declared when $P \leq 0.05$. Compared with LMO herds, HMO herds fed more NDF (20.87% and 42.10%, respectively) and ADF (17.82% and 38.32%, respectively, $P < 0.05$). HO herds classified as HMO produced more milk fat than LMO herds (3.90 v. 3.56%, respectively, $P < 0.05$). FA content was not affected by body condition, stocking rate, or lameness ($P > 0.05$). The relationship between FA content and milk fat varies with breed and nutrition. Milk FA, particularly DNFA and MOFA, could be used as predictors of disorders or stressors to the cow.

Key Words: de novo, milk fat

102 Milk fat and protein yield in Holstein California herds with different milk production levels. M. B. Abreu*[1,2], M. I. Marcondes[1], F. C. Ferreira[2], B. Verboort[3], and N. Silva-Del-Río[2], [1]*Universidade Federal de Vicosa*, *Vicosa, MG, Brazil*, [2]*Veterinary Medicine Teaching and Research Center, University of California-Davis*, *Tulare, CA*, [3]*Agritech Analytics*, *Visalia, CA*.

The objective of the present study was to benchmark milk components (fat and protein) across Holstein California dairy herds with different milk production levels (MPL). Dairy Herd Improvement Association records from 2017 were obtained from Agritech Analytics (Visalia, CA). The initial data set included information from 343 herds, 572,893 cows, and 3,182,862 milk tests. Data was screened using proc Means of SAS. The effect of MPL on milk components was evaluated using the GLIMMIX of SAS. Least squares means were considered different when $P \leq 0.05$. The final data set included herds with ≥200 cows and >5 mo test, and cows with until 200 d in milk (DIM). Cow observations with <2% or >6% of fat and protein content, and milk yield ≥ 10 kg were considered outliers. After data screening, the final data set include 238 herds, 462,550 cows, and 1,830,884 milk tests. Herds were classified according to MPL as low (LP; 1: < 32 kg; n = 59), medium (MP; $Q_1 \leq$ to $\leq Q_3$: 32 ≤ to ≤38 kg; n = 118), and high (HP; $> Q_3$: > 38 kg; n = 61) production level. On average, the final enrolled herds had 2,763, 2,365, and 2,882 cows and produced 31, 39, 43 kg/d respectively for LP, MP, and HP. Milk fat (%) [4.39% (LP); 3.64% (MP); 3.63%(HP)] and milk protein (%) [3.47%(LP) 3.07%(MP); 3.02%(HP)] significantly decreased as MPL increased. However, average milk fat yield (kg/cow/d) [1.31 (LP); 1.40 (MP); 1.58 (HP)] and protein yield (kg/cow/d) [1.03 (LP); 1.19 (MP); 1.31 (HP)] significantly increased as MPL increased. There was a significant effect of the month and a month by MPL interaction for milk fat and protein (%). For the top 10 fat and protein producing herds (9 HP, 1 MP), fat per cow ranged from 1.66 to 1.84 kg/d (3.46 to 3.86%) and protein per cow 1.37 to 1.59 kg/d (2.93 to 3.24%) respectively. In conclusion, although LP herds had the greatest association with milk fat and protein content, HP herds showed greatest association with milk fat and protein yield.

Key Words: milk production level, milk components, California dairies

103 Do biological and management causes of a short or long dry period induce the same effects on dairy cattle productivity? K. E. Olagaray*[1], M. W. Overton[2], and B. J. Bradford[1], [1]*Kansas State University*, *Manhattan, KS*, [2]*Elanco Animal Health*, *Greenfield, IN*.

A retrospective observational study utilized 32,182 lactations from 16 farms to determine if management versus biological reasons for a short or long dry period have the same associations with subsequent lactation performance. Herd inclusion criteria were Holstein cows, herd size ≥900 cows, breeding by artificial insemination, and bimonthly milk testing. Dry period (DP) length and gestation length (GL) were each categorized as short (>1 SD below mean) or long (>1 SD above mean) and combined to generate the following 7 study groups: short DP, short GL (S_DS_G); short DP, average GL (S_DA_G); average DP, short GL (A_DS_G); average DP, average GL (A_DA_G); average DP, long GL (A_DL_G); long DP, average GL (L_DA_G); and long DP, long GL (L_DL_G). Continuous data were analyzed by mixed models and time to event data by Cox proportional hazard models, both accounting for clustering at the herd level. First test and whole lactation milk and component yields were least for S_DS_G. Within cows that experienced calving difficulty, time to first service was delayed 13 and 20% for S_DS_G and A_DS_G compared with A_DA_G. Hazard of leaving the herd by 60 DIM was 34% greater for A_DS_G than A_DA_G. Similar outcomes between S_DS_G and A_DS_G but not S_DA_G indicated short GL was a greater contributor to poor performance than DP length itself. Overall production was similar between A_DA_G and S_DA_G; however, first test somatic cell linear score was greater and milk yield was lesser for S_DA_G cows with greater milk at last test before dry-off. Long DP or GL did not impact early lactation or whole lactation milk yield. Cows with a long DP due to management factors (L_DA_G) likely experienced issues related to excessive lipid mobilization as milk fat concentration and fat:protein ratio at first test were greater and hazard of leaving the herd was 30 and 24% greater compared with A_DA_G by 60 and 365 DIM, respectively. In conclusion, deviations in DP length caused by biology (short GL) were associated with greater impacts than management causes of short DP, whereas management reasons for long DP were associated with more negative outcomes than long GL.

Key Words: gestation length, lactation, survival

104 Economics of capture of phosphorus from liquid dairy manure. J. Harrison*[1], K. Fullerton[1], E. Whitefield[1], K. Bowers[2], and S. Norberg[3], [1]*Washington State University*, *Puyallup, WA*, [2]*Multiform Harvest*, *Seattle, WA*, [3]*Washington State University*, *Pasco, WA*.

A project was conducted with the goal of developing a nutrient recycling relationship between the dairy producers and alfalfa forage growers in Washington State. A mobile fluidized-bed cone (32,000 L) was used to evaluate the capture of P in the form of struvite (magnesium-ammonium phosphate) from undigested and anaerobically digested liquid dairy manure. Manure from ~30 dairies was evaluated and In 27 runs when reduction in ortho-P was positive, the average reduction in ortho- P was 32% with a range of 1 to 76%. The greatest capture rates were achieved with anaerobically digested manure since more of the P is in an inorganic form and captured in struvite. The factors that affected performance were: % suspended solids, Calcium, Fe, ratio of ortho-phosphate-P:total

P, and ammonia concentration. When using data from the highest recoveries of P, the cost as calculated on a per cow per d basis when considering chemical costs were: $0.22 (anaerobically digested manure) to $0.39 (undigested manure). The use of a cost per cow per d metric for cost is not the most appropriate way to evaluate the cost to a given farm since the goal is to achieve a net zero balance of P imports and exports from any given farm. The net zero balance will be affected by factors such as: number of cows, number of acres utilized for manure application, phosphorus utilization by crops grown, double or triple cropping strategies, diet manipulation, and manure export off-farm. The factor having the greatest impact on achieving P balance is the land base for growing crops that utilize manure. When using the scenario of 1000 cows, 67 g per d per cow excreted P, and 27 kg P uptake by crops per year, the annual cost to achieve balance for respective hectares were: anaerobically digested manure 242 ha, $275,074; 283 ha, $182,693; 324 ha, $90,312; 344 ha, $44,122, and 362 ha, $2550. The annual cost to achieve balance for respective acres were: undigested manure 242 ha, $523,208; 283 ha, $347,494; 324 ha, $171,779; 344 ha, $83,922, and 362 ha, $4,851.

Key Words: manure, phosphorus, nutrient balance

106 Economic impacts of feeding an immune modulator to multiparous dry Holstein dairy cows. L. T. Casarotto*[1], V. Ouellet[1], J. Laporta[1], J. D. Chapman[2], A. De Vries[1], and G. E. Dahl[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL*, [2]*Phibro Animal Health Corporation*, *Teaneck, NJ.*

Feeding OmniGen-AF (OMN, Phibro Animal Health) to lactating and dry cows exposed to heat stress lowers respiration rates and rectal temperatures and improves lactational performance and health. Our aim was to evaluate the effects of feeding OMN beginning at dry off until mid-lactation and quantify the economic impact of this supplementation strategy. Cows in a commercial Holstein dairy herd were randomly assigned to OMN (56 g/d, n = 706) or control (CON; 56 g/d of placebo, n = 686) supplementation from dry off (~60 d before calving) to 150 DIM (~210 d total feeding length). Milk yield, reproductive performance, and health events were analyzed using MIXED and LIFETEST procedures of SAS. Milk yield of OMN cows was 0.7 kg/d greater than CON (40.8 kg/d vs. 40.1 kg/d respectively; $P < 0.01$). Using a milk price of $0.44/kg and a lactation length of 150 DIM, income from milk would be increased $46/cow when OMN is fed. During lactation, OMN feeding tended ($P < 0.11$) to reduce the incidence of mastitis (257 vs. 284 cases), retained placenta (38 vs. 52 cases), displaced abomasum (18 vs. 22 cases) and days in hospital relative to CON. Considering the costs of treatment (i.e., labor, medications, veterinarian fees, and replacement costs) and the number of cases of each disorder, feeding OMN reduced health-associated costs by $14/cow relative to CON. Feeding OMN also improved reproductive performance as reflected in a 10-d reduction in days open ($P < 0.05$) compared with CON. Assuming an extra day open cost of $3/d, the enhanced reproductive performance associated with feeding OMN would lead to a savings of $30/cow compared with CON. Ultimately, total economic gain associated with OMN supplementation for 210d was of $90/cow. The cost of supplementation was $32/cow, for a net benefit of $58/cow. In conclusion, our results indicate that OmniGen-AF supplementation from dry-off through 150 DIM benefits cow health and performance and improves herd profitability.

Key Words: dairy economics, milk, reproduction

Ruminant Nutrition: Gut Physiology, Fermentation, and Digestion

107 The effect of 3-nitrooxypropanol on ruminal microbial gene expression in dairy cows. D. Pitta*[1], A. Melgar[2], N. Indugu[1], V. Shabtai[1], M. Hennessy[1], B. Vecchiarelli[1], M. Kindermann[3], N. Walker[3], R. Thauer[4], and A. Hristov[3], [1]*University of Pennsylvania School of Veterinary Medicine*, *Kennett Square, PA*, [2]*The Pennsylvania State University*, *University Park, PA*, [3]*DSM Nutritional Products*, *Basel, Switzerland*, [4]*Max Planck Institute for Terrestrial Microbiology*, *Marburg, Germany*.

Research indicates that 3-nitrooxypropanol (3-NOP), a methane inhibitor under investigation, reduces enteric methane emissions by an average of about 24% in dairy cows. However, studies on the contribution of different methanogens to methanogenesis in the rumen and the effect on these pathways by 3-NOP are lacking. The purpose of this experiment was to investigate the effect of 3-NOP on inhibition of different methanogenesis pathways in dairy cows fed silage-based diets over a 12-week period using metatranscriptomics. Rumen contents of 8 ruminally-cannulated early-lactation dairy cows were sampled 2 h after feeding during wk 4, 8, and 12 of a randomized complete block design experiment in which 3-NOP was fed at 60 mg/kg feed dry matter intake. Cows (4 control and 4 3-NOP) were blocked based on their previous lactation milk yield or predicted milk yield. Rumen samples were extracted for RNA and reverse transcribed to cDNA. Libraries were then constructed, sequenced on the Illumina HiSeq platform, and sequences were annotated against the KEGG database. Three methanogenesis pathways were identified: the hydrogenotrophic ($CO_2 + H_2$) pathway was the predominant pathway (contributing to about 70% of methanogenesis in the rumen in this experiment), followed by the methanol-utilizing (15%) and methylamine-utilizing pathways (10%). Six species of *Methanobrevibacter*, 2 species of *Methanosphaera,* and *Methanomassiliicoccales* representatives were involved in the hydrogenotrophic pathway, methanol-reducing, and methylamine-reducing methanogenesis pathways, respectively. The most abundant archaeal transcripts were those of genes encoding the α, β, and gamma chains of methyl co-enzyme reductase (MCR), an enzyme that facilitates the formation of methane and is present in all methanogens. This study showed that 3-NOP numerically reduced MCR transcripts by 4, 30, and 12% at wk 4, 8, and 12, respectively, accompanied by a 25% reduction in enteric methane (g/kg ECM, $P < 0.0001$). It can be concluded that inhibition of the MCR enzyme in ruminal methanogens is responsible for the reported reduction in enteric methane emission by 3-NOP.

Key Words: enteric methane, rumen microbe, dairy cow

108 Temporal changes in total and metabolically active ruminal methanogens induced by 3-nitrooxypropanol in dairy cows. D. Pitta[1], A. Melgar[2], N. Indugu[1], C. Pappalardo*[1], M. Hennessy[2], B. Vecchiarelli[1], V. Shabtai[1], M. Kindermann[3], N. Walker[3], and A. Hristov[2], [1]*University of Pennsylvania School of Veterinary Medicine*, *Kennett Square, PA*, [2]*The Pennsylvania State University*, *University Park, PA*, [3]*DSM Nutritional Products*, *Basel, Switzerland*.

It has been shown that enteric methane production in dairy cows peaks within 6 h post-feeding and that the mitigating effect of 3-nitrooxypropanol (3-NOP), a methane inhibitor under investigation, is highest immediately post-feeding and lowest before feeding. The effect of 3-NOP on methanogen populations in the rumen over the course of the day has not been investigated. The purpose of this study was to investigate the effect of 3-NOP on total and metabolically active methanogens in the rumen of dairy cows over the course of the day and over a 12-week period. Rumen contents of 8 ruminally-cannulated early-lactation dairy cows were sampled at 2, 6, and 10 h after feeding during wk 4, 8, and 12 of a randomized complete block design experiment in which 3-NOP was fed at 60 mg/kg feed dry matter intake. Cows (4 control and 4 3-NOP) were blocked based on their previous lactation milk yield or predicted milk yield. Rumen samples were extracted for microbial DNA (total) and microbial RNA (metabolically active), PCR-amplified for the 16S rDNA gene of archaea, sequenced on an Illumina platform, and analyzed for archaea diversity. There was a difference ($P < 0.05$) between DNA and cDNA-based archaea communities revealing that methanogens have different metabolic capacities. At the community level, weighted UniFrac analysis (commonly present populations) revealed differences ($P < 0.05$) by treatment, week, and their interaction. In the unweighted UniFrac analysis (presence-absence information), methanogen communities differed by treatment, week, sampling hour, and their interaction in both DNA and cDNA-based analysis. *Methanobrevibacter* was the dominant genus followed by *Methanosphaera*, with the latter genus having greater abundance in the metabolically active component compared with total populations. The relative abundance of *Methanosphaera* was higher at 2 h compared with 6 and 10 h after feeding, and the reverse was true for *Methanobrevibacter*. These findings show that *Methanosphaera* may increase following feed intake and may have a greater share in total methane formation than previously thought.

Key Words: enteric methane, methanogen diversity, dairy cow

109 Early life modulation of the gut microbiota and antibiotic resistance in calves fed oregano essential oil. P. P. Ray*[1], C. Rymer[1], D. Wilde[2], E. F. Lund[2], and A. C. Singer[3], [1]*Department of Animal Sciences, School of Agriculture, Policy and Development, University of Reading*, *Reading, United Kingdom*, [2]*Anpario plc*, *Worksop, United Kingdom*, [3]*NERC Centre for Ecology and Hydrology*, *Wallingford, United Kingdom*.

This study aimed to investigate the modulation in the gut microbiota and associated changes in antibiotic resistance in calves fed Oregano essential oil (EO). Ten Holstein bull calves were divided into 2 groups (5 calves/group) and assigned to 1 of 2 dietary treatments: control (fed waste milk) and EO (Orego-Stim Liquid, Anpario plc, UK; fed waste milk with EO for the first 10 d of the study) within 48 h after birth. Fecal grab samples were collected on d 0, 3, 10, 21 and at weaning. Replica plating technique was used to determine the proportion of *E. coli* colonies resistant to a 4th generation cephalosporin (cefquinome). Genomic DNA was extracted, 16S rRNA (V3-V4) was amplified and sequenced using Illumina MiSeq platform. Data were processed in R using DADA2 Pipeline and analyzed using Minitab. Firmicutes and Actinobacteria were 2 most predominant phyla across all samples. While fecal abundance of Firmicutes was higher in control calves compared with EO-fed calves (66 vs 44%; $P = 0.01$), Actinobacteria was more abundant in EO-fed calves than in control calves (39 vs 15%; $P = 0.05$). Relative abundance of genus *Butyricicoccus* in the feces was higher in control calves compared with EO-fed calves (19 vs 9%; $P = 0.04$). *Arthrobacter* and *Escherichia* tended to be less abundant (3.8 vs 3.9%; $P = 0.10$; 0.4 vs 0.6%; $P = 0.05$) and *Ruminococcus* tended to be more abundant in EO-fed calves compared with control calves (2.1 vs 1.5%; $P = 0.05$). Feeding EO did not influence overall species richness and evenness. However, both richness and evenness were greater in control calves compared with EO-fed calves on d 3 and 10, but not after d 10. Relative abundance of *Arthrobacter* was positively correlated to the fecal abundance of cefquinome resistant *E. coli* ($\rho = 0.56$; $P < 0.01$). Fecal count of *Cryptosporidium* eggs tended to increase with increasing abundance of *Arthrobacter* ($\rho = 0.40$; $P = 0.09$) and decreasing abundance of *Ruminococcus* ($\rho = 0.40$; $P = 0.10$). Feeding EO to young calves could modulate the gut microbiota such that growth of antibiotic resistant bacteria and parasite colonization is reduced.

Key Words: calves, antibiotic resistance, essential oil

110 Improving rumen microbial fermentation profile with natural active ingredients. R. Temmar[1], M. E. Rodriguez-Prado*[1], G. Forgeard[2], C. Rougier[2], and S. Calsamiglia[1], [1]*Animal Nutrition and Welfare Service, Univeristat Autonoma de Bar-*

celona, *Bellaterra, Spain*, [2]*Techna France Nutrition*, *Coueron, France*.

Different doses of essential oils were screened to select those able to increase propionic and/or butyric and decrease acetic acids using an in vitro batch fermentation system. Treatments were control (CTR), monensin (MON, at 1.25 and 12.5 mg/L) and 13 essential oils from: cassia, anise star, geraniol, lemongrass, limonene D-natural and thyme (at 80, 300 and 750 mg/L); tea tree and coriander seed (at 40, 150 and 375 mg/L); capsicum oleoresin and black pepper (at 0.4, 3 and 7.5 mg/L); garlic (at 30, 120 and 300 mg/L); and turmeric and ginger (at 10, 40 and 150 mg/L). Treatments were added to 50 mL of a 1:1 buffer: rumen fluid medium with 0.5g of a 50:50 forage to concentrate diet under anaerobic conditions and starting pH of 6.6. The experiment was done in triplicate in 2 independent periods. After 24 h, samples were taken for pH, volatile fatty acids and ammonia-N concentration determinations. Effects were analyzed using the PROC MIXED procedure of SAS. The majority of essential oils had no effect at low doses except for garlic and lemongrass. Garlic at 30, 120 and 300 mg/L tended to increase ($P < 0.10$) propionate and decreased ($P < 0.05$) the acetate-to-propionate ratio. Lemongrass at 80 mg/L tended to increase propionate ($P < 0.10$) and decreased ($P < 0.05$) the acetate-to-propionate ratio. Anise star and thyme ($P < 0.05$) at 300 mg/L and coriander seed at 150 mg/L ($P < 0.10$) increased the proportion of butyrate. Capsicum at 3 mg/L, coriander seed at 150 mg/L and thyme at 750 mg/L decreased ($P < 0.05$) ammonia-N concentration, but the rest of essential oils did not change the ammonia-N concentration. Results confirm the ability of some essential oils to modulate microbial fermentation in a direction consistent with higher propionate molar proportion, and lower acetate molar proportion, the acetate-to-propionate ratio and ammonia-N concentration without changing total VFA in effective doses between 30 and 750 mg/L depending on essential oils.

Key Words: essential oils, rumen microbial fermentation

111 Exploring synergistic interaction between essential oils in in vitro rumen microbial fermentation. R. Temmar[1], M. E. Rodriguez-Prado*[1], G. Forgeard[2], C. Rougier[2], and S. Calsamiglia[1], [1]*Animal Nutrition and Welfare Service, Universität Autonoma de Barcelona*, *Bellaterra, Spain*, [2]*TECHNA France Nutrition*, *Coueron, France*.

Mixtures of lemongrass (LEM), coriander seed (COR) and ginger oils (GIN) were screened to explore synergistic effects using an in vitro batch fermentation system. Incubations were conducted in a 1:1 rumen fluid: buffer media with 0.5 g of a 50:50 forage to concentrate diet at a starting pH of 6.6, 38°C and anaerobic conditions. Incubations were done in triplicate and in 2 periods. After 24 h, samples were taken for pH, volatile fatty acids (VFA) and ammonia-N concentration determinations. Doses were: LEM (50, 100 mg/L), COR (50, 100 mg/L) and GIN (75, 150 mg/L) mixed at different proportions. Treatments were: T1 (100% LEM), T2 (100% COR), T3 (100% GIN), T4 (50% LEM, 50% COR), T5 (50% LEM and 50% GIN), T6 (50% COR, 50% GIN), T7 (33% LEM, 33% COR, 33% GIN), T8 (50% LEM, 25% COR, 25% GIN), T9 (25% LEM, 50% COR, 25% GIN), T10 (25% LEM, 25% COR, 50% GIN), T11 (100% LEM, 100% COR, 100% GIN), control (CTR), monensin at 10 mg/L (MON) and a blank. All treatments were diluted in ethanol, and the control and blank were also dosed with the equivalent amount of ethanol (0.2 mL). Using LEM, COR and GIN individually did not modify microbial fermentation. However, mixing together LEM and GIN at 62.5 and 125 mg/L increased ($P < 0.05$) propionate molar proportion and tended ($P < 0.10$) to decrease acetate molar proportion and the acetate-to-propionate ratio without changing total VFA and ammonia-N concentration, resulting in a synergistic effect. Mixing together COR and GIN at 62.5 and 125 mg/L increased ($P < 0.05$) butyrate molar proportion, decreased ($P < 0.05$) acetate and tended to decrease ($P < 0.1$) the acetate-to-propionate ratio without changing total VFA and ammonia-N concentration, also resulting in synergistic effect. Careful selection and combination of these EO may result in useful mixtures with synergistic interactions to modulate rumen microbial fermentation.

Key Words: essential oils mixtures, rumen microbial fermentation, synergies

Small Ruminant

112 Effect of protected dietary oils on dry matter intake, nutrient digestibility, and milk production in dairy goats. E. Vargas-Bello-Pérez*[1], R. Ayala-Hernández[2], N. Pescador-Salas[2], O. Castelán-Ortega[2], and M. González-Ronquillo[2], [1]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen*, *Frederiksberg C, Denmark*, [2]*Facultad de Medicina Veterinaria y Zootecnia, Instituto Literario 100, Universidad Autónoma del Estado de México*, *Toluca, Estado de México, México*.

This study evaluated the effect of protected dietary oils on dry matter intake (DMI), digestibility and milk production in dairy goats. Nine Saanen goats with 150 d in milk were blocked by live weight (LW; 59 ± 4 kg) and used in a 3 × 3 Latin square design (n = 3) with 25-d periods and were kept in individual pens. Periods consisted of 17 of diet adaptation and 8 d of data collection. A basal diet based on barley hay and corn silage was supplemented with 2.7% DM of calcium soaps of either palm (PO), canola (CO) or safflower (SO) oils. Goats were milked daily at 0700h, and milk yield was recorded daily in all experimental periods. Data for LW, dry matter intake (DMI), nutrient digestibility and milk production was analyzed using the MIXED procedure of SAS. Sums of squares from the data were separated into overall mean, goat (within square), square, period, diet, and overall error. All variables were considered fixed, except for goat (within square) and overall error, which were considered random. DMI was higher ($P < 0.001$) in PO and CO than in SO (113 and 112 vs. 87 g/kg $LW^{0.75}$). Compared with CO, in vivo digestibilities of dry matter (65.3 ± 2.25 vs. 55.2 ± 2.39 and 58.8 ± 2.32%), organic matter (66.3 ± 2.23 vs. 55.9 ± 2.39 and 60.6 ± 1.90%), and neutral detergent fiber (61.6 ± 2.73 vs. 46.0 ± 6.14 and 51.0 ± 4.27%) were lowered ($P < 0.001$) by SO and PO. Compared with PO and CO, milk production increased ($P < 0.001$) by SO (0.88 ± 0.19 and 0.95 ± 0.11 vs. 1.10 ± 0.17 kg/d). Overall, compared with PO and SO, CO promoted a more efficient use of nutrients in dairy goat diets.

Key Words: goat, oils, milk

113 Effect of dietary inclusion of sunflower seed silage (*Helianthus annuus*) on physical and sensory characteristics of sheep cheese. A. C. Narvaes-López[1], L. E. Robles-Jiménez[1], E. Cardoso-Gutiérrez[1], M. d. l. Á. Colín-Cruz[1], M. González-Ronquillo[1], and E. Vargas-Bello-Pérez*[2], [1]*Facultad de Medicina Veterinaria y Zootecnia, Instituto Literario 100, Universidad Autónoma del Estado de México*, *Toluca, Estado de México, México*, [2]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen*, *Frederiksberg C, Denmark*.

The objective of this study was to evaluate the physical and sensory characteristics of cheeses from sheep supplemented with sunflower seed silage (SFS). Six East Friesian ewes were used in a 3 × 3 Latin square design during 3 periods of 20 d. At the beginning of the study animals were 70 ± 5 d in milk. Animals were kept in individual pens. Experimental periods consisted of 15 d for diet adaptation and the last 5 d for milk collection. Animals received a control diet (25%DM corn silage, 25%DM alfalfa hay, 50%DM concentrate), and supplemented diets with whole sunflower seed (SF; 5%DM) or SFS (5% DM). Two cheeses per period per treatment (1 kg/each) were manufactured and allowed to ripen for 180 d. After ripening times, an acceptance test was performed with a panel composed of 50 untrained judges. Evaluations on odor, color, texture, taste and overall acceptability used a 5-point hedonic scale (1 = lowest and 5 = highest; intensities). Likewise, pH, colorimetric and quantification of mesophiles, coliforms, fungi and yeasts were determined. Data were analyzed in a completely randomized design using GLM procedure form SAS. No differences were observed in odor (3.08 ± 0.20), color (3.46 ± 0.38) and texture (3.31 ± 0.58). Scores for taste were higher ($P < 0.001$) in SF and overall acceptability was lower ($P < 0.001$) in SFS. Compared with control, pH was lower ($P < 0.05$) in SFS (5.31 ± 0.03 vs. 4.87 ± 0.06). Lightness (L*; 71.0 ± 2.27), redness (a*; −0.79 ± 0.78), yellowness (b*; 20.9 ± 1.04), chroma (C*; 20.96 ± 0.75) and hue (H*; 92.3 ± 2.15) were similar between treatments ($P > 0.05$). Counts for mesophiles, coliforms, fungi and yeasts were similar between treatments ($P > 0.05$). Results showed that SFS decreases overall acceptability of ripened cheeses without detrimental effects on color and microorganism presence.

Key Words: sheep, cheese, sunflower

114 Effect of dietary neutral detergent fiber on mid-infrared predicted milk constituents of non-traditional dairy sheep breeds milked on an accelerated lambing system. N. Kochendoerfer*, M. L. Thonney, and D. M. Barbano, *Cornell University*, *Ithaca, NY*.

The effect of replacing dietary NFC with highly digestible NDF from soy hulls (SH) on milk constituents of Finnsheep × Dorset ewes (n = 53) was tested in short and frequent 73-d lactations. The experiment was a triply replicated Latin square (3 ewe groups each with 3 pens of 4–6 animals per pen, 3 lactations, 3 diets). The diets (L-SH, M-SH, H-SH) contained 31.7, 36.3, 40.0% aNDFom and 41.3, 37.2, 31.9% NFC, respectively. Milk was weighed at each 2X milking and ~9 weekly samples across each of the 127 lactations were collected and refrigerated until analyzed. The 1170 samples were analyzed with a Fourier transform mid-infrared spectrophotometer and validated with Mojonnier ether extract, Kjeldahl, and MUN reference chemistry. Milk yield and component concentrations were regressed on DIM to obtain average concentrations for each individual lactation. The resulting data were analyzed using a model that included Square Number, Pen and Lactation within Square Number, and Diet. True protein, fat, and lactose concentrations, with means of 5.28 ± 0.05, 5.91 ± 0.09, and 4.81 ± 0.08, respectively, were not significantly affected by level of dietary NDF. True protein, fat, and lactose yields were highest for ewes fed the M-SH diet ($P < 0.05$), reflecting their overall higher milk yields. Ewes fed the M-SH diet generated the highest concentrations of de novo, mixed, and preformed fatty acids per 100 g milk ($P < 0.05$). Lactose predicted milk yield ($r^2 = 0.99$) and the relationship between protein and milk yield was stronger ($r^2 = 0.93$) than the relationship between fat and milk yield ($r^2 = 0.75$). Mixed origin fatty acids had the strongest relationship with total fat concentration ($r^2 = 0.71$). Percentages of fatty acids that were de novo and mixed origin had means of 38.5 ± 0.27, and 26.7 ± 0.19, respectively, and were not affected by diet. Preformed fatty acid concentrations were highest ($P < 0.05$) for the M-SH diet (35.5 ± 0.16). The diet with medium soyhull inclusion supported significantly higher milk and milk component yields, as well as numerically higher milk fat concentrations.

Key Words: sheep, sheep milk, NDF

115 A high rumen degradable starch diet modulates jejunum microbiota and alters enterohepatic circulation of bile acids in dairy goats. J. Shen[1], X. Chen[2], L. Wang[1,2], J. Yao[1], and Y. Cao*[1,2], [1]*Northwest A&F University*, *Yangling, Shaanxi, China*, [2]*Harvard Medical School*, *Boston, MA*.

The aim of this study was to investigate the mechanism of milk fat depression in the liver and small intestine of dairy goats fed different rumen degradable starch (RDS) diets. Eighteen Guanzhong lactating goats (2nd lactation, 45.8 ± 1.54 kg) in experiment 1 and 6 ruminally cannulated dairy goats (aged 2–3 y, 54 ± 2.4 kg) in experiment 2 were used to investigate the effect of dietary RDS content (LRDS = 20.52%, MRDS = 22.15%, HRDS = 24.88%) on liver metabolism and free-LPS concentrations, respectively. The treatments lasted for 28 d with 21 d for adaption and 7 d for sampling. In experiment 1, blood samples were collected for analyses of biochemical parameters; liver, ileal mucosa, and jejunal contents samples were collected for analysis of metabolites, mRNA expression and microbiota, respectively. In experiment 2, rumen fluid and blood samples were collected for determination of free-LPS. Comparisons were made between the treatments using Student's *t*-test. Free-LPS concentrations were analyzed using the MIXED procedure of SAS. Compared with LRDS, the HRDS significantly decreased the milk fat composition (3.74% to 3.01%), enhanced the activity of alkaline phosphatase and aspartate aminotransferase in plasma, increased free-LPS concentrations in both rumen liquid (7570 EU/mL to13600 EU/mL) and plasma (0.037 EU/mL to 0.179 EU/mL) ($P < 0.05$), indicating hepatic injury were induced by HRDS. HRDS significantly decreased the concentrations of certain fatty acids (included linolenic acid, etc.), and phospholipids (including PC (18:0/18:1), etc.), and increased the concentrations of L-carnitine and L-palmitoylcarnitine (VIP > 1, $P < 0.10$), and the protein expression of carnitine palmitoyltransferase-1 ($P < 0.05$) in liver compared with the LRDS, indicating HRDS promoted lipid β-oxidation. Compared with LRDS, HRDS significantly increased the relative abundance of *Firmicutes* and *Ruminococcus_2* in jejunal content ($P < 0.05$, LDA > 2), increased the concentrations of taurochenodeoxycholate and taurodeoxycholic acid (VIP > 1, $P < 0.10$) and mRNA expression of *CYP7A1* ($P < 0.05$) in liver, decreased the expression of bile acid receptor *FXR* ($P < 0.05$) in ileum mucosa, indicating the negative feedback regulation of bile acid synthesis was inhibited. This study demonstrated that HRDS feeding modulates jejunum microbiota and alters enterohepatic circulation of bile acids, and promotes lipid β-oxidation in dairy goats.

Key Words: bile acids, enterohepatic circulation, lipid metabolism

116 The effects of *Saccharomyces cerevisiae*-based products on the ruminal environment and performance of sheep submitted to dietary changes.

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The increase in the use of energy foods in ruminant can trigger important metabolic disturbances, making the use of additives an effective strategy in modulating the ruminal environment with performance optimization. The aim of the study was to evaluate the effects of *Saccharomyces cerevisiae* in maintaining ruminal pH, performance and metabolism in sheep submitted to changes in the diet starch concentration. For this purpose, 20 adult female sheep were used in a confined system and randomized assigned to 1 of 3 treatments: no yeast (Control; n = 6), yeast culture (YC; n = 7) and hydrolyzed yeast (HY; n = 7) supplemented with 5g/sheep/day. The groups were submitted to 4 experimental periods of 5 d, varying the composition of the diet in 40 and 60% wheat bran and the remainder composed of roughage based on corn silage. The diet in periods 1 and 3 was composed by 40% of wheat bran and periods 2 and 4 by 60% of wheat bran on DM basis. Feed offered was adjusted to achieve 3% of BW. Blood, ruminal fluid and BW were evaluated at the end of each period and the DMI was recorded daily. Ruminal fluid was analyzed to determine the physical parameters, ruminal pH, bacterial activity through the methylene blue test and evaluation of the motility of the protozoa under an optical microscope immediately after collection. Data were analyzed using NCSS software and the averages were analyzed using the mixed models method. Although rumen pH was not affected ($P > 0.05$) by treatment, protozoan motility was more efficient in supplemented animals ($P < 0.05$). The DMI was not affected, however, there was a trend to increase the daily gain (kg/d) in YC group in comparison with control in the third period (0.47 vs. 0.11 ± 0.26 kg) ($P = 0.10$). The HY showed an increase in total proteins ($P < 0.01$) in the last 2 periods in comparison with others, indicating a greater supply of available protein and a better adaptation to dietary changes, even with higher concentration of starch. The aspartate aminotransferase enzyme, an important indicator of liver damage, was greater ($P < 0.01$) in HY fed animals in the second period in comparison with control, which was maintained in the following period (97.7 vs. 80.1 ± 6.6 U/L). Yeast supplementation seems to be a potential tool when diet changes are necessary, partially by improving the performance of confined sheep.

Key Words: yeast culture, ruminal modulation, starch

117 Metabolic and productive characteristics of sensitive and heat-tolerant phenotypes of Murciano-Granadina dairy goats.

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Thirty-three Murciano-Granadina does in mid-lactation (2.45 ± 0.14 kg/d milk; 40.9 ± 1 kg BW) were submitted to a short-term challenge (2 h) passing from thermo neutral (TN, THI = 65) to heat stress (HS, THI = 86) conditions. Variation of thermophysiological traits (RT, rectal temperature; RR, respiratory rate) were used to calculate the change ratio (CR = Σ after/before) and to select does differing in tolerance (TO, tolerant, 4.20 ± 0.21, n = 9; SE, sensitive, 6.25 ± 0.24, n = 10; $P < 0.01$). The does were allocated to a crossover of 2 periods (12-d each) with 2 climatic conditions: TN (15 to 20°C; THI = 63–65), and 2) HS (30 to 37°C; THI = 88–79). RT, RR and lactational traits were recorded daily, whereas milk and blood samples were collected weekly. At the end of each period a glucose tolerance test (GTT; glucose, 0.15 g/kg BW; samples, min –15 to 120) was done. The HS does, increased RT 0.82°C (0.58 and 1.05°C a.m. and p.m. respectively; $P < 0.001$), RR 224% (164 and 285%, a.m. and p.m. respectively; $P < 0.001$) and water consumption (74%; $P < 0.001$), whereas decreased feed intake (–10%; $P < 0.001$), in comparison to TN. Milk yield increased by 5% ($P < 0.005$) but energy-corrected milk (2.0 ± 0.1 kg/d) did not vary by HS. Milk fat (–16%), protein (–14%) and lactose (–4%) contents decreased ($P < 0.01$) by HS. Blood glucose, insulin, and urea did not change, but HS goats had huge PRL and greater creatinine (819 and 14%, respectively; $P < 0.01$) than TN. Blood insulin did not vary between TN and HS does, but glucose disposal was greater in HS. Comparing TO and SE phenotypes, no differences were detected in RR, but p.m. RT was lower in the SE goats. No differences were detected in feed and water intakes, milk yield and composition, as well as in basal blood metabolites. AUC glucose at 60 min tended to be lower ($P < 0.07$) in SE than in TO goats. In conclusion, Murciano-Granadina goats, in terms of thermophysiological and lactational traits and blood metabolites, were moderately affected by HS. Responses to GTT indicated that HS increased insulin resistance and that TO and SE goats employed different mechanisms to keep blood glucose levels under HS conditions.

Key Words: heat tolerance, challenge, dairy goat

Breeding and Genetics

118 Association between lifetime selection index predictions and lifetime performance. B. Fessenden[1], D. Weigel[2], J. Osterstock[1], D. Galligan[3], and F. Di Croce*[1], [1]*Zoetis Genetics*, *Kalamazoo, MI*, [2]*Zoetis Outcomes Research*, *Kalamazoo, MI*, [3]*University of Pennsylvania School of Veterinary Medicine, New Bolton Center*, *Kennett Square, PA*.

Selection indexes are critical to genetic improvement as they combine values for many traits into a single value that can be used to rank animals and inform breeding decisions. In 2016, Zoetis Genetics developed the Dairy Wellness Profit Index (DWP$) to estimate the genetic potential for lifetime profit in US Holsteins. This study's goal was to evaluate if selection index genomically enhanced predicted transmitting abilities had the capability to predict observed lifetime profit in US Holstein animals. Five large herds (n = 2,175 enrolled cows) in the United States were chosen for this study because they had: 1) genomic predictions from females born in 2011, 2) recorded production, reproduction and health events to accurately estimate profit per cow, and 3) at least 200 animals born in 2011. Selection index predictions from 2012 were used to rank the 2,175 animals within herd and assign cows to percentile-based DWP$ groups (genetic groups: Worst 25%, 26–50%, 51–75%, and Best 25%). Herd records were used to calculate lifetime energy-corrected milk, income over feed cost, and lifetime days in milk based on the actual performance from first freshening through when they left the herd; for cows that were still in the herd, current totals were used. Analysis results indicated that DWP$ predictions were associated with differences in phenotypic lifetime energy-corrected milk, income over feed cost, and lifetime days in milk between the worst and best genetic groups. The difference between the worst and best genetic groups was 9004 kg lifetime energy-corrected milk ($P < 0.01$), $1,607 income over feed cost ($P < 0.01$), and 202 d in milk ($P < 0.01$). These results demonstrate that a lifetime selection index can in fact predict differences in actual lifetime profit potential of individuals. These results indicate that DWP$ predictions of young calves and heifers can be used to make informed predictions of future lifetime performance.

Key Words: selection indexes, genomics, lifetime profit

119 Conformation traits of Holstein cows and their association with the Pro$ selection index. L. Alcantara*[1], C. Baes[1,2], G. Oliveira Junior[1], and F. Schenkel[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada*, [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern*, *Bern, Switzerland*.

Breeding programs across the globe are shifting their selection goals from production-driven to more balanced indexes that include traits of direct and indirect economic significance, such as conformation, fertility and health. In Canada, for instance, changes were made to better reflect producers and market needs in the wake of improved accuracy of genetic and genomic evaluations and national efforts regarding data recordings. In 2015 a new national profit index, Pro$, was introduced to maximize production yields, while maintaining functional and conformation traits, taking into consideration the accumulated profit to 6 years of age of proven sire's daughters. The Canadian classification system comprises several linear and nonlinear descriptive traits and defective characteristics that are taken into account to calculate points, which are distributed in 4 different scorecard sections: Mammary System (MS), Feet & Legs, Dairy Strength (DS) and Rump. Understanding the contribution of each of these traits to a monetary index, such as Pro$, would help producers make culling and mating decisions ultimately resulting in more profitable herds. Therefore, the present study used statistical regression and principal component analysis to look at the overall and individual contribution of 23 classification traits to the Pro$ index using deregressed Estimated Breeding Values from 9,292 proven bulls (Lactanet). The total variance explained by the full model was 0.66, and out of the traits included in the model, Dairy Capacity contributed the most to the increase in Pro$. Stepwise backward regression analysis indicated that all traits should remain in the full model. Principal component analysis showed that the vast majority of traits contributed positively to Pro$ values. Traits from the MS contributed the most to the increase in Pro$ values, whereas Thurl Placement and Rump Angle did not seem to have a sizeable impact on Pro$.

Key Words: conformation traits, Pro$, Holstein

120 Opportunity costs in beef-on-dairy breeding strategies. A. De Vries*, *University of Florida*, *Gainesville, FL*.

The use of beef-on-dairy is growing, but the most profitable breeding strategy is often not clear. The objectives were to (1) quantify the value of breeding strategies, (2) discover most profitable, but sometimes complex breeding strategies, and (3) determine the opportunity costs from more constrained or simpler breeding strategies. A daily Markov Chain model including heifers and at least 5 parities of cows was developed. The distribution of predicted transmitting abilities for an economic selection index within the herd depended on genetic reliabilities, genetic trend and genetic variances. Unrestricted breeding opportunities allowed for any combination of sexed, conventional and beef semen. Inputs regarding reproduction, milk production, forced culling, and prices mimicked an average herd and allowed for a calculation of profit/milking cow per yr. A nonlinear solver optimized breeding strategies subject to constraints such as the number of dairy heifer calves to be at least equal to the number needed to replace culled cows. Surplus dairy heifer calves with the lowest predicted transmitting abilities were sold. Using genomic reliabilities and typical market prices of calves, the optimal breeding strategy used a combination of sexed, conventional, and beef semen throughout the herd. Older cows received less sexed semen and more beef semen. Crossbred calves were 34% of all calves born. This strategy made 16% surplus dairy heifer calves. Limiting surplus to 0% reduced profit by $13/milking cow per yr. Limiting surplus to 0% and not using conventional semen reduced profit by $24/milking cow per yr. Use of only conventional semen reduced profit by $63/milking cow per yr. This value is partitioned as $64 greater operational net revenues, $15 lower semen costs, $61 greater genetic lag costs, $50 lower calf sales, and $1 lower genomic testing costs. Opportunity costs for simple strategies ranged from $36 to $72/milking cow per yr. Use of traditional reliabilities resulted in lower opportunity costs for non-optimal strategies. In conclusion, optimal beef-on-dairy breeding strategies can be complex and opportunity costs from simpler strategies may be substantial.

Key Words: beef-on-dairy, opportunity cost, genetic lag

121 The effect of synchronized breeding on genetic evaluations of fertility traits in dairy cattle: Preliminary analysis. C. Lynch*[1], G. A. Oliveira Junior[1], F. S. Schenkel[1], and C. F. Baes[1,2], [1]*Centre for Genetic Improvement of Livestock, University of Guelph*, *Guelph, ON, Canada*, [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern*, *Bern, Switzerland*.

Hormonal synchronization alleviates the pressure of estrus detection by affecting the estrous cycle, allowing for higher rates of conception even for naturally low fertile animals. This leads to genetically inferior cows' performance becoming masked and potentially resulting in similar performance to that of naturally fertile animals. As genetic programs rely on the collection of accurate phenotypic data, phenotypes recorded on treated animals likely affect the genetic evaluations. This study analyzed the traits calving to first service (CTFS), first service to conception and days open in 419,102 cows from 1122 herds with 1,811,394 breeding and fertility records across 3 scenarios. Scenario 1 compared records that included any type of hormone treatment (HORM) against all other records. Scenario 2 compared HORM vs heat detection protocol (HD) records. Scenario 3 compared hormonal synchronization protocol (FTAI) records vs HD. Available data for each trait was characterized based on the use of hormone treatment, for example CTFS was split whereby one subset included only FTAI records while the second data set included only HD records. Preliminary statistics are presented in Table 1. By conducting a bivariate analysis the results of this study will identify potential genetic and non genetic differences between the fertility phenotypes recorded under the different scenarios. In addition, this will provide a novel basis in assessing the impact of hormonal synchronization protocols on the accuracy of genetic evaluations for fertility traits in dairy cattle.

Key Words: fertility traits, hormonal synchronization, genetic evaluations

122 Implementation of national health trait evaluations in Jersey. K. L. Parker Gaddis*[1], L. M. Jensen[2], P. M. VanRaden[3], J. H. Megonigal Jr.[1], E. L. Nicolazzi[1], H. D. Norman[1], and C. W. Wolfe[4], [1]*Council on Dairy Cattle Breeding*, *Bowie, MD*, [2]*University of Florida*, *Gainesville, FL*, [3]*Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA*, *Beltsville, MD*, [4]*American Jersey Cattle Association*, *Reynoldsburg, OH*.

Health evaluations for 6 traits (resistance to milk fever, displaced abomasum, ketosis, mastitis, metritis, retained placenta) have been available for Holstein animals from the Council on Dairy Cattle Breeding (CDCB; Bowie, MD) since April 2018. Recent research indicated that expanding these evaluations to include Jersey animals was feasible. Concurrently, there was a 112% increase in the total number of usable Jersey health records submitted to CDCB in the span of one year beginning in January 2019. Total number of available phenotypic records by trait as of January 2020 ranged from 85,417 for ketosis to 168,342 for mastitis. Overall incidence ranged from 1.2% for milk fever up to 10.4% for mastitis, similar to those found in Holstein. Heritabilities were assumed to be equivalent to those in Holstein, ranging from 0.6 to 3.1%. The same pipelines as those currently used for CDCB Holstein health evaluations were expanded to include Jersey data. Phenotypes are pre-adjusted for unequal variance before evaluation. Traditional PTA are estimated using a univariate BLUP repeatability animal model accounting for year-season, age-parity, herd-year, and permanent environmental effects, as well as a regression on inbreeding and heterosis. Genomic PTA are calculated with 79,294 markers used in CDCB routine genomic evaluations. Resulting PTA are presented as percentage points above or below the breed's average resistance with more positive values being favorable. Average traditional reliabilities for bulls born since 1990 with ≥90% net merit (NM$) reliability ranged from 17 to 32%, depending on trait. Average genomic reliabilities for those bulls ranged from 29 to 49%, gaining 12 to 17 percentage points from the inclusion of genomic data. Maximum PTA reliability was 98% for mastitis. Correlations between health PTA and PTA of other routinely evaluated traits were calculated. Significant ($P < 0.05$) correlations ranged from −0.52 between mastitis and milk up to 0.33 between displaced abomasum and livability. Beginning with the April 2020 CDCB evaluations, Jersey animals will receive evaluations for all 6 health traits. The 6 health traits in NM$ will receive 2% emphasis.

Key Words: health, Jersey, national evaluation

123 Breeding dairy cattle for the future: Where is the Canadian industry headed? S. Larmer*, *Semex Alliance*, *Arthur, ON, Canada*.

Recent uptake of both genomic technology, improved herd fertility, and advanced reproductive technologies including IVF and sexed semen have drastically shifted the focus of dairy cattle breeders globally. This session will explore how these technologies can be used in tandem both in Canadian dairy herds and globally to maximize the potential productivity and efficiency of dairy cattle on commercial dairy farms. Specifically, we will explore the future of breeding cattle with a focus on the productivity of those animals, rather than a focus specifically on the additive genetic potential of the next generation. This includes the estimation and use of higher order genetic terms and better methods of genomic inbreeding to maximize expected production in the next generation. In the Canadian industry, as is seen globally, we see a clear shift away from the traditional model of data sharing between producer and the public evaluation provider, often stemming from a perception that milk recording systems do not provide enough proportional value to the farm. This is especially driven by the rapid growth in robotic milking systems, where production and other performance metrics are available to the farm on a 24x7 basis. This provides an exceptional challenge for the overall industry to maintain and enhance genetic and genomic predictions for economically important traits. The future will need to adapt to this growing reality, including a shift towards accessing, standardizing and utilizing data from these robotic systems, as well as designing new traits where reference populations can be established to effectively and accurately measure data on a smaller, representative group of animals ("closer to biology" traits). Finally, we will look at the future of indexes that drive profitability on farm, and the impact that creating the right index for a specific production system can have for the productivity and profitability of a dairy producer, using examples from Canada and globally.

Breeding and Genetics

124 Genomic prediction of dairy bull fertility using X chromosome markers. H. A. Pacheco*[1], F. M. Rezende[1,2], and F. Peñagaricano[1], [1]*University of Florida*, *Gainesville, FL*, [2]*Universidade Federal de Uberlândia*, *Uberlândia, MG, Brazil*.

Service sire has been recognized as an important factor affecting dairy herd fertility. Our group has reported promising results on genomic prediction of dairy bull fertility using autosomal SNP markers. Little is known, however, about the genetic contribution of sex chromosomes, which are enriched in genes related to sexual development and reproduction. As such, our goal was to evaluate the genomic prediction of service sire fertility including SNP markers on the X chromosome (BTAX). Data set consisted of 5,014 Holstein bulls with sire conception rate records and genotypes for roughly 291k SNP located on the autosomes, 1.5k SNP located on the pseudoautosomal region (PAR), and 13.7k X-specific SNP. The analyses included linear and Gaussian kernel-based models fitting either only SNP markers on autosomes (291k SNPs) or combining autosomal with PAR and X-specific SNP markers (291k SNPs + 15.2k SNPs). Predictive performance was evaluated using 10-fold cross-validation repeated 10 times. Interestingly, the linear kernel model that included the BTAX markers exhibited a higher average predictive correlation than the linear kernel model that fitted only autosomal SNPs (0.338 vs 0.319). The prediction of bull fertility was further improved by using a Gaussian kernel-based model combining autosomal and BTAX SNPs. Indeed, this model fitting markers on 'autosomes + BTAX' delivered an average predictive correlation equal to 0.343, representing an increase in predictive ability of about 7.5% compared with the standard whole autosomal genome model. Overall, our findings indicate that including markers on the X chromosome would benefit the genomic prediction of bull fertility. These results may help to improve conception rates in dairy herds through accurate genome-guided decisions on bull fertility.

Key Words: kernel-based prediction models, sex chromosomes, sire conception rate

125 Variation in the timing of puberty in grazing Holstein-Friesian dairy heifers. N. Steele*[1], M. Stephen[1], A. Young[1], B. Kuhn-Sherlock[1], P. Amer[2], S. Meier[1], C. Phyn[1], and C. Burke[1], [1]*DairyNZ Limited, Private Bag 3221*, *Hamilton, New Zealand*, [2]*AbacusBio Limited*, *Dunedin, New Zealand*.

Age at puberty varies widely across and within herds, with research indicating that timing of puberty could be an early predictor of cow reproduction. Commercial dairy herds (n = 54) from 3 regions in New Zealand were studied to investigate the phenotypic and genetic (co)variances of age at puberty and measures of fertility in Holstein-Friesian dairy cattle. Herein, we report preliminary phenotypic findings of the timing of puberty and associated BW in 5,010 heifers born in spring 2018. Herds were visited on 3 occasions to collect blood samples when average (±SD) heifer ages for each herd were 299 ± 15 d (visit 1; V1), 327 ± 15 d (V2) and 355 ± 15 d (V3). Body weights were measured at V2. Blood plasma was assayed to determine progesterone (P4) concentration, and the timing of puberty was assigned to each animal as either V1, V2, or V3 when their P4 reached a threshold of ≥1 ng/mL for the first time. A heifer was considered still pre-pubertal after V3, if P4 concentration did not exceed this threshold in any collected samples. We identified that 19.4% (n = 973) of heifers had reached puberty by V1 (average age and BW ± SD; 302 ± 14 d and 245 ± 33 kg), 18.8% (n = 941) by V2 (331 ± 16 d and 239 ± 32 kg), 16.8% (n = 842) by V3 (355 ± 14 d and 229 ± 31 kg), and 43.3% (n = 2,170) had not reached puberty by V3 (352 ± 14 d and 211 ± 30 kg). A further 84 heifers (1.7%) were not assigned an age at puberty due to missing blood samples. A large variation was observed between herds in the percentage of heifers reaching puberty (mean = 61% ± 25% of heifers by V3, range 2% to 100%). Research into management factors contributing to between-herd variation is underway. Next, we will examine the phenotypic variation in puberty onset between animals that can be explained by genetics. Relationships between puberty and subsequent fertility measures during lactation will then be investigated to identify earlier, novel traits to estimate genetic merit for fertility.

Key Words: pubertal age, body weight

126 Alternative models for genetic analysis of pregnancy loss in dairy cattle. A. Sigdel*, R. S. Bisinotto, and

Table 1 (Abstr. 125). Preliminary statistics of data

		Scenario Groups			
Trait	Stats	OTHER (n=1,404,752)	HORM (n=406,642)	HD (n=763,567)	FTAI (n=273,733)
Calving to first service, d	Mean	80.46	79.05	77.09	78.82
	Range	230	230	230	230
	SD	28.24	22.32	25.36	20.99
First service to conception, d	Mean	54.77	60.24	55.9	62.49
	Range	205	205	205	205
	SD	52.99	55.2	52.68	55.94
Days open	Mean	161.48	160.84	158.12	160.23
	Range	388	388	386	383
	SD	75.35	73.93	73.7	73.12

F. Peñagaricano, *University of Florida*, *Gainesville, FL.*

Pregnancy loss directly impairs reproductive performance in dairy cattle. Early pregnancy losses (before 30 d after AI) cannot be detected and are indistinguishable from conception failure. Here, we evaluated the loss of pregnancy following accurate detection of a viable embryo. As such, our objectives were to assess alternative models for genetic analysis of presence (BIN) or number (NUM) of pregnancy losses in US Holstein cows. Linear and Probit models were fitted for BIN, whereas linear and Poisson models were used for NUM. Data consisted of 14k confirmed pregnancy/abortion records on 8k Holstein cows distributed over the first 2 lactations. All models included days in milk, year-season, and types of service (insemination or embryo transfer) as fixed effects, and animal and service sire as random effects. The alternative models were compared with respect to goodness-of-fit, ranking of sires, and predictive ability in 5-fold cross-validation. Estimates of heritability ranged from 1% to 8% for BIN and 1% to 9% for NUM. Nonlinear models (Probit and Poisson) showed better goodness-of-fit than their counterpart linear models. From a breeder's perspective, an important question is whether these models yield different breeding decisions. The Spearman rank correlations between bulls' breeding values were high, from 0.84 to 0.97, suggesting a minor re-ranking. All the models exhibited similar predictive ability. Indeed, for BIN models, the mean-squared error of prediction (MSEP) ranged from 0.16 to 0.18, whereas for NUM models, MSEP values ranged from 0.14 to 0.16. Overall, our results suggest that pregnancy loss is a heritable trait, and hence, genetic selection for reduced risk of abortion is feasible. In addition, the use of nonlinear models seems a reasonable choice for analyzing pregnancy losses.

Key Words: heritability, non-linear models, reproductive performance

127 Targeted sequencing reveals deleterious mutations affecting dairy bull fertility. R. Abdollahi-Arpanahi*, H. A. Pacheco, and F. Peñagaricano, *University of Florida*, *Gainesville, FL.*

Bull fertility is often overlooked as a potential cause of reproductive inefficiency in dairy cattle. However, semen from one bull is used to inseminate hundreds of cows, and hence, one subfertile bull could have a major impact on herd reproductive performance. We previously identified 5 genomic regions, located on BTA8 (72.2 Mb), BTA9 (43.7 Mb), BTA13 (60.2 Mb), BTA17 (63.3 Mb), and BTA27 (34.7 Mb), that show very significant dominance effects on dairy bull fertility. Each of these regions explains about 5–8% of the observed differences in sire conception rate between bulls. Here, we aimed to identify the causal variants responsible for this variation using targeted sequencing (10Mb per region). For each genomic region, 2 DNA pools were constructed from high-fertility and low-fertility Holstein bulls. The DNA Sequencing analysis included reads quality control (using FastQC), genome alignment (using BWA and ARS-UCD1.2), variant calling (using GATK) and variant annotation (using Ensembl). The sequencing depth per pool varied from 39X to 51X. We identified 7,144 SNPs with opposing homozygous genotypes between low- and high-fertility pools. Notably, 74 of these SNPs were annotated as missense mutations, and at least 5 of them were classified as strong candidate causal variants, i.e., missense mutations with deleterious effects located on genes exclusively/highly expressed in testis or actively involved in the fertilization process. These candidate causal mutations are located in genes *ADAM28*, *TTLL9*, *TCHP*, *FOXN4* and *ADAM2*. This work is the foundation for the development of novel genomic tools for improving dairy bull fertility, these causal mutations will allow the early detection and culling of subfertile bull calves or young genomic bulls.

Key Words: causal variants, DNA sequencing, sire conception rate

128 Multiparous Holstein cow vaginal microbiome near parturition associated with neonatal fecal microbiome. C. E. Owens*, H. G. Huffard, A. I. Nin-Velez, A. J. Duncan, C. L. Teets, K. M. Daniels, K. F. Knowlton, and R. R. Cockrum, *Virginia Polytechnic Institute and State University*, *Blacksburg, VA.*

Initial microbial inoculation of the calf gut is thought to stem from the dam's birth canal and colostrum; the objective of this study was to identify interrelationships between dam reproductive, colostrum, and calf fecal microbiomes. Multiparous Holstein cows (n = 6) were enrolled at 14 d before expected calving. At 10 d before expected calving, a calving alert system was placed on cows. Flocked swabs of the posterior vagina were collected within 24 h before calving. At birth, calves (n = 6; heifers = 3, bulls = 3) were immediately isolated and meconium samples were collected. Representative colostrum samples were collected within 1 h of calving and representative placenta samples were collected within 6 h of calving. Calf fecal samples were collected at 24 h and 7 d of age. Bacterial DNA was isolated from all samples and 16S rDNA amplicons underwent 2 × 300 paired end sequencing on the Illumina MiSeq platform. Sequences were aligned to the 97% Greengenes reference database in CLC Genomics Workbench. Alpha diversity was calculated using phylogenetic diversity and β diversity was calculated using weighted unifrac distances. Spearman's rank correlations were performed based on genera relative abundance in the placenta, vagina, and colostrum of a dam and its calf's meconium and fecal samples. Proteobacteria were the most abundant phylum in the placenta (48%), vagina (58%), colostrum (96%), and 24 h calf feces (85%), while Bacteroidetes were the most abundant phylum in meconium (43%) and 7 d calf feces (43%). Colostrum and placenta samples had the least phylogenetic diversity within each sample, but had a high similarity between samples. Genera in the vagina had a moderate correlation with genera in meconium ($r_s = 0.45 \pm 0.03$). Genera in colostrum had a low correlation with 24 h calf feces ($r_s = 0.10 \pm 0.04$). Dam vaginal microbiota could be used to predict calf gut composition. Further research on understanding these relationships could lead to discovery of core microbes that are the most influential on microbial composition.

Key Words: microbiome, calf, reproduction

129 Investigating conception rate for beef service sires bred to dairy cows. T. M. McWhorter*[1], J. L. Hutchison[2], H. D. Norman[3], J. B. Cole[2], G. C. Fok[3], D. A. L. Lourenco[1], and P. M. VanRaden[2], [1]*Department of Animal and Dairy Science, University of Georgia*, *Athens, GA*, [2]*USDA Animal Genomics and Improvement Laboratory*, *Beltsville, MD*, [3]*Council on Dairy Cattle Breeding*, *Bowie, MD.*

The widespread use of sexed semen on US dairy cows has led to an excess of replacement heifers' calves, and the sale prices for those calves are much lower than in the past. Cows not selected to produce the next generation of replacement heifers are increasingly being bred to beef bulls to produce crossbred calves for beef production. The purpose of this study was to investigate the use of beef service sires bred to dairy cows and heifers and to provide a tool for dairy producers to evaluate beef service sires' conception. Sire conception rate (SCR) is a phenotypic evaluation of service sire fertility that is routinely calculated for US dairy bulls. A total of 268,174 breedings were available which included 36 recognized beef breeds and 7 dairy breeds. Most of the beef-on-dairy inseminations (95.4%) were to Angus (AN) bulls; therefore, final evaluations were restricted to AN service sires bred to Holstein (HO) cows. Pedigree data for AN bulls was unavailable. There were 233,379 breedings from 1,344 AN service-sire to 163,919 HO cows. A mean (SD) conception rate of 33.8% (47.3%) was observed compared with 34.3% (47.5%) for breedings with HO sires mated to HO cows. Mean SCR reliability was 64.5% for 116 publishable bulls, with a maximum reliability 99% based on 25,217 breedings. Average SCR was near 0 (on AN base) with a range of −5.1 to 4.4. Breedings to HO heifers were also examined which included 19,437 breedings (443 AN service sire and 15,971 HO heifers). A mean (SD) conception rate of 53.0% (49.9%) was observed, compared with 55.3% (49.7%) for breedings with a HO sire mated to a HO heifer. Beef sires were used more frequently in cows known to be problem breeders, which explains some of the difference in CR. Mean service number was 1.92 and 2.87 for HO heifers, and 2.13 and 3.04 for HO cows mated to HO and AN sires, respectively. Mating dairy cows to beef bulls may be profitable if calf prices are higher, fertility is improved, or if practices such as sexed semen, genomic testing, and improved cow productive life allow herd owners to produce both higher quality dairy replacement and increased income from market calves.

Key Words: sire conception rate, beef-on-dairy, beef bull fertility

130 Across-country genomic prediction of bull fertility in Jersey dairy cattle. F. M. Rezende*[1], M. Haile-Mariam[2], J. E. Pryce[2], and F. Peñagaricano[1], [1]*University of Florida*, *Gainesville, FL*, [2]*Agriculture Victoria Research*, *Bundoora, VIC, Australia*.

The use of information across populations is an attractive approach to increase the accuracy of genomic predictions for numerically small breeds and traits that are time-consuming and difficult to measure, such as male fertility in cattle. This study was conducted to evaluate genomic prediction of Jersey bull fertility using an across-country reference population combining records from United States (US) and Australia (AU). Data set consisted of 1.5k US Jersey bulls with sire conception rate (SCR) records, 603 AU Jersey bulls with semen fertility value (SFV) records and roughly 90k SNP genotypes. Both SCR and SFV are evaluations of service sire fertility based on cow field data, and both are intended as phenotypic evaluations because the estimates include genetic and non-genetic effects. Within and across-country genomic predictions were evaluated using univariate and bivariate GBLUP models. Predictive ability was assessed in 5-fold cross-validation using the correlation between observed and predicted fertility values. Genomic predictions within-country exhibited predictive correlations around 0.30 and 0.02 for US and AU, respectively. The AU Jersey population is genetically diverse, so careful selection of the reference population by including only closely related animals (e.g., excluding New Zealand bulls) allowed to increase the predictive correlations up to 0.20. Notably, the use of bivariate models fitting all US Jersey records and the optimized AU population allowed to achieve predictive correlations around 0.24 for SFV values, which is a gaining in predictive ability of 20%. Conversely, for predicting SCR values, the use of an across-country reference population did not outperform the standard approach using a pure US Jersey reference data set. Overall, our findings indicate that genomic prediction of male fertility in cattle is feasible, and the use of an across-country reference population would be beneficial when local populations are small and genetically diverse.

Key Words: multi-country reference population, semen fertility value, sire conception rate

Dairy Foods Processing

131 Effect of pH on the morphology and tensile properties of milk protein-based electrospun fibers. S. Akkurt and P. Tomasula*, *Dairy and Functional Foods Research Unit, USDA, Agricultural Research Service, Eastern Regional Research Center*, *Wyndmoor, PA.*

Electrospinning has been used to produce edible mats from nanosized fibers of calcium (CaCN) or sodium (NaCN) caseinate with pullulan(PUL) added to facilitate fiber formation. The electrospun mats are a promising method to preserve and deliver bioactives to foods; e.g., because of the fiber's large surface area/volume, but fiber imperfections such as bead formation and variations in diameter size would prevent consistent performance of the mats. To improve fiber quality, NaCl has been recommended as an additive to increase shear viscosity for drawing thinner fibers while electrospinning; however, we found NaCl ineffective and hypothesized that NaOH would increase solution viscosity and also strengthen the mats through CN aggregate formation. The objective of this study was to investigate the effect of pH on the morphology and tensile properties of CaCN or NaCN with PUL electrospun fibers. Aqueous solutions of 15 (wt/wt)% CaCN or NaCN, and PUL were prepared at 20°C. Then, 1:1(pH 6.7) and 2:1(pH 6.7) blends of either CN with PUL solution were prepared and adjusted from the initial pH6.7 to pH 8, 9, and 10 with 1N NaOH. A syringe fed 3 mL of each solution to an electrospinning unit at flow rate of 3mL/h and at 20kV, with fibers deposited on a rotating drum. At pH6.7, the mean diameters, D, of the CaCN and NaCN:PUL(1:1) fibers were 226 ± 25 and 215 ± 18nm, respectively, with beads noted on the fibers. As pH was increased to 8 and then 9, the fibers became thicker with D of 274 ± 20 and 234 ± 16nm and bead-free, becoming thinner at pH 10, with D of 105 ± 16 and 93 ± 15nm, respectively. Similar trends were noted for the (2:1) blends. Tensile Strength (TS) and Elongation at Break% (EAB) measurements showed that the initial (TS,EAB%), (1.4 ± 0.5MPa, 4.0 ± 0.6%) and peak (4.7 ± 0.3MPa, 8.4%) values the for CaCN mats occurred at pH 9 with a sudden 60% decline in these values at pH10 suggesting that the negatively-charged CN aggregates partly disassociated. This did not occur for the NaCN aggregates at pH10 most likely due to lower electrostatic repulsion. Studies on CN in fibers will provide insights to their structure and that relative to casein micelles.

Key Words: nanofibers, caseinates, fibrous mats

132 Production of high-purity phospholipid concentrate from buttermilk powder using ethanol-modified supercritical carbon dioxide. A. Ubeyitogullari* and S. S. H. Rizvi, *Cornell University*, *Ithaca, NY.*

Milk fat globule membrane (MFGM) phospholipids are in considerable demand due to their well-known health benefits and superior functionality to create clean-label products. However, MFGM phospholipid concentrates of commercial utility are still scarce. This study was designed to explore a new strategy using ethanol-modified supercritical carbon dioxide (SC-CO_2) to produce MFGM phospholipid concentrate of high purity. The effects of extraction conditions (temperature, pressure, and ethanol concentration) on the yield and phospholipid concentration were investigated. The total lipid and phospholipid contents of commercially available buttermilk powder were determined using the conventional Folch extraction and found to contain 9.0% total lipids, of which 60% was phospholipids. A sequential extraction strategy of using pure SC-CO_2 followed by ethanol-modified SC-CO_2 was employed to first extract the nonpolar lipids as the first fraction and the phospholipid-rich concentrate was obtained as the second fraction. The total lipid yield of the first fraction was 2.5% which contained only 0.3% phospholipids. On the other hand, the second fraction yielded 4.3% total lipids with a phospholipid content of 76%, which, to the best of our knowledge, is the highest phospholipid concentration obtained from buttermilk powder. The sequential SC-CO_2 extraction process achieved a recovery of 97% of the nonpolar lipids and 61% of the phospholipids from buttermilk powder. At the optimized extraction conditions, the major phospholipids in the extract were dihydrosphingomyelin (DHSM; 5%), sphingomyelin (SM; 24%), phosphatidylethanolamine (PE; 22%), phosphatidylserine (PS; 2%), phosphatidylinositol (PI; 3%), and phosphatidylcholine (PC; 44%). The triacylglycerol compositions of the extracts obtained by Folch and SC-CO_2 extractions methods were similar. Such phospholipid-rich concentrate offers a new opportunity for value addition to buttermilk powder using a commercially scalable process.

Key Words: buttermilk, phospholipids, supercritical carbon dioxide

133 Understanding the switchable solvent extraction of phospholipids from dairy by-products. K. Rathnakumar* and S. I. Martinez-Monteagudo, *South Dakota State University*, *Brookings, SD.*

Switchable solvents have been used for the extraction and subsequent separation of polar compounds. These solvents represent a new class of solvents having the capacity to change their polarity with an external stimulus. Recently, we have used a tertiary amine (N,N-dimethylcyclohexylamine, CyNMe2) as a switchable hydrophilicity solvent (SHS) for extracting phospholipids from different dairy matrices. The underlying principle behind the change in polarity is due to the formation of ammonium carbonate salt in the presence of CO_2. Upon removing the CO_2 with nitrogen, the carbonate salt returns back to its original amine. Unfortunately, the extraction mechanism by which CyNMe2 acts is largely unknown, which may limit its further development. In this work, we extracted PLs from buttermilk and B-serum by CyNMe2. In addition, the extraction was systematically studied in terms of yield of PLs, gel electrophoresis, particle size, scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM). For comparison, the PLs were also extracted using Folch extraction. For buttermilk, the extraction of PLs by CyNMe2 resulted in yields remarkably higher (significantly different $P < 0.05$ according to Tukey's test) than the control treatment (99.96 ± 1.21 and 11.37 ± 2.31%, respectively). Similarly, the recovered PLs in B-serum by CyNMe2 (7.57 ± 0.59%) were significantly different ($P < 0.05$ according to Tukey's test) by then the control extraction (5.34 ± 0.61%). Analysis of gel electrophoresis revealed some degree of disruption of the protein matrix, being more extensive in the case of SHS. Images through CLSM of untreated samples showed aggregates of fat and protein entrapped, while the use of CyNMe2 resulted in disruption of such aggregates. Additionally, SEM images revealed the separation of the lipids from the protein network with the extraction by CyNMe2. Our results indicate that the extraction of PLs from buttermilk and B-serum is mainly ascribed to protein disruption, which may release the PLs into the aqueous medium. The outcomes of this study help to gain insights into the extraction mechanism by which CyNMe2 acts, and develop extraction strategies for protein matrices.

Key Words: tertiary amine, phospholipids, byproducts

134 Pilot-scale production of enriched phospholipid concentrate from whey protein phospholipid concentrate using enzymatic hydrolysis and microfiltration. A. V. Swaminathan*[1], M. Molitor[2], K. J. Burrington[2], D. Otter[2], and J. A. Lucey[1,2], [1]*University of Wisconsin-Madison*, *Madison, WI*, [2]*Wisconsin Center for Dairy Research*, *Madison, WI.*

Whey protein phospholipid concentrate (WPPC) contains high amounts of phospholipids (PL) (3–5% db) thereby making it a good source for further extraction of PL to make highly enriched PL product. Enzymatic hydrolysis in combination with microfiltration (MF) was used for pilot-scale production of an enriched PL concentrate derived from WPPC. This was achieved by allowing hydrolyzed peptides to pass through into MF permeate while concentrating fat and PL in MF retentate. Benchtop experiments were done to determine the most suitable proteolytic enzyme from among 5 different commercial proteases. SDS-PAGE analysis was done to measure the extent of protein hydrolysis over a period of 4 h with samples drawn every 30 min. Alcalase enzyme was found to exhibit maximum proteolytic activity at optimum conditions of pH 8 and temperature of 50°C. The intensity of major protein bands (MFGM, caseins, β-lactoglobulin) was found to decrease in SDS-PAGE profiles as hydrolysis progressed, along with bands appearing below 17 kDa. This indicated that degradation of proteins resulted in the generation of smaller peptides. Pilot-scale MF production coupled with diafiltration (DF) yielded a final retentate with total PL content of 9.3% (% of final retentate in db) and with protein and fat contents at about 43.8 ± 0.4% (db) and 48.9 ± 1.2% (db), respectively. A 2-fold increase in PL content (db) was therefore achieved through this process with an ~18% reduction in protein content. The MF permeate had 0% fat content indicating that there was no transmission of lipids or phospholipids through the permeate during MF/DF process. Complete removal of proteins and peptides was not achieved by this process; either additional enzymes or more extensive DF might be needed to remove more peptides.

Key Words: whey protein phospholipid concentrate, phospholipids, microfiltration

Production, Management, and the Environment

135 Evaluating the predictive ability of point cloud deep learning to identify individual animals using surface-based body shape of dairy calves. R. E. P. Ferreira*[1], T. Bresolin[1], L. G. Pereira[2,1], and J. R. R. Dorea[1], [1]*University of Wisconsin-Madison*, *Madison, WI*, [2]*Embrapa Dairy Cattle*, *Juíz de Fora, MG, Brazil*.

Advances in machine learning techniques have allowed the development of computer vision systems (CVS) that can potentially monitor growth development of livestock animals. In this context, depth images from dorsal view are the most used inputs to estimate animal BW and biometrics. Frequently, such CVS rely on identification (ID) systems as a way to match animal ID and predicted phenotype. However, the use of surface-based body shape acquired from dorsal images to predict BW could also be adopted for animal recognition. Such alternative would optimize CVS to recognize animal ID and monitor growth development at the same time. Nonetheless, growing animals are continuously changing body shape, which could limit its use as an invariant feature for pattern recognition. Thus, the objectives of this study were: (1) to investigate the use of 3D dorsal images from calves as a potential tool for animal identification; and (2) to investigate if changes in body shape due to growth affect the prediction accuracy for animal identification. Images from 5 Holstein calves were acquired over 3 weeks using a depth sensor. From each image, the background was removed and only 3D data points of the animal's back were used. For objective 1, the algorithm was trained (n = 111), validated (n = 23) and tested (n = 22) using images within wk 1. For objective 2, the algorithm was trained (n = 221) and validated (n = 53) using images from wk 1 and 2, and tested using images from wk 3 (n = 172). Accuracy within wk 1 was 90.9% on the testing set. In objective 2, the overall accuracy decreased to 54.7% on the testing set. The prediction accuracy for each respective animal was: 38.5% (n = 32); 97.1% (n = 55); 88.4% (n = 62); 3.1% (n = 38); and 32.4% (n = 34). Animals with reduced number of images in the training data set presented lower accuracies on the testing set. Thus, increasing the amount of training samples can potentially improve predictive performance when later weeks are evaluated. The results show that the use of animal's 3D body surface is a promising tool for animal recognition.

Key Words: growth, calf, deep learning

136 Inconsistency of precision dairy monitoring technologies measuring the same behaviors. B. W. Jones*[1,2], I. C. Tsai[3], Y. M. Chang[4], and J. M. Bewley[5], [1]*Tarleton State University*, *Stephenville, TX*, [2]*Texas A&M AgriLife Research*, *Stephenville, TX*, [3]*DeLaval*, *Lansing, MI*, [4]*University of London*, *London, UK*, [5]*CowFocused Solutions*, *Elizabethtown, KY*.

The objective of this study was to evaluate agreement among precision dairy monitoring technologies (PDMT) measuring same behaviors. Holstein cows (n = 108) were fitted with PDMT 10d before their impending calving date. Six different PDMT, namely, IceQube (IQ; IceRobotics Ltd., Edinburgh, Scotland), AfiAct Pedometer Plus (AFI; Afimilk, Kibbutz Afikim, Israel), Track a Cow (TAC; ENGS Systems Innovative Dairy Solutions, Israel), Smartbow (SB; Smartbow GmbH, Jutogasse, Austria), CowScout S Leg (GEA; GEA Farm Technologies GmbH, Bönen, Germany) and Cow Manager SensOor (SEN; Agis Automatisering, Harmelen, Netherlands) were included in the study. Six different behaviors, lying time (hours/day), step (number/day), lying bouts (number/day), activity (hours/day), eating time (hours/day), and rumination (hours/day), were recorded. Before analysis, data was cleaned by removing potential outliers. Spearman's correlation was used to evaluate rank correlation among PDMT. All analyses were carried out in SAS (Cary, NC). For lying time, AFI, IQ and TAC show strong correlations (r_s ranged from 0.79 to 0.90), and they were weakly correlated with SB (r_s ranged from 0.33 to 0.43). For number of steps, GEA was moderately correlated with all other technologies (r_s ranged from 0.50 to 0.61) and all other technologies were only weakly correlated (r_s ranged from 0.39 to 0.47). Weak to moderate correlations among lying bouts measures existed (r_s ranged from 0.42 to 0.66). Correlations between 2 activity measures and the 2 eating time measures were low (r_s = 0.32 and 0.37, respectively). Moderate correlation for rumination time was observed between SEN and SB (r_s = 0.61). Overall, there is limited agreement among most PDMT except for lying time. Although PDMT offers exciting opportunities to develop automated monitoring products to inform farm management, quality of captured data requires careful scrutiny.

Key Words: precision dairy monitoring technology, accelerometer

137 Is the rate of milk yield increase in early lactation associated with milking frequency, rumination, and activity in dairy cows? M. Peiter*, H. N. Phillips, and M. I. Endres, *Department of Animal Science, University of Minnesota*, *Saint Paul, MN*.

Peak milk yield (PMY) has been shown to be positively associated with whole lactation milk yield. Cows that peak early with high PMY and are able to maintain a high overall milk yield during their lactation are desirable. Therefore, the objectives of this study were: 1) to investigate the association between milk yield increase rate (MIR) and 250-d milk yield (250MY), and 2) to investigate the association between MIR and early lactation change in rumination time (RT), activity, and number of successful milkings. Daily cow data were collected from 34 free-flow AMS farms in MN and WI. Milk yield increase rate was calculated by dividing PMY by days at peak for each cow. A simple linear regression line of best fit was estimated for RT over the first 7 DIM for each cow to find the change in RT. Activity and milkings were averaged over the first 7 DIM. A nonparametric regression test using Huh-Jhun methods while controlling for parity group was performed to measure the association between MIR and 250MY. A multivariate linear mixed model was performed with MIR as the outcome variable (n = 3,117). The model included main effects of change in RT, activity, milkings, and parity (1, 2, 3+) as fixed effects. Farm and cow within farm were included as random effects. The Wald statistic indicated that the 250MY increased by 455 kg per each kg increase in MIR (n = 859; P = 0.0002). The inclusion of all the predictors improved the multivariate linear mixed model fit. However, milkings had the greatest association with MIR (0.067 ± 0.007 kg/d per 1-unit increase in milkings; $P < 0.0001$). The mean [95% CI] MIR was 0.76 [0.72, 0.81], 1.33 [1.3, 1.4], and 1.38 [1.3, 1.4] kg/d for parity 1, 2, and 3+, respectively. Parities 2 and 3+ had greater MIR than parity 1 ($P < 0.0001$). Due to the association found between the rate of increase in milk yield until peak and 250MY yield, this study suggests that increasing the number of milkings during the first 7 DIM may increase the MIR and subsequent whole lactation milk yield.

Key Words: early lactation, milking frequency, milk yield

138 Energy consumption on five Midwest dairy farms. K. Sharpe*, B. Heins, E. Buchanan, and M. Reese, *West Central Research and Outreach Center*, *Morris, MN*.

The objective of this study was to measure electricity use from specific components on 5 Midwest dairy farms. Data were collected from farms located in west central Minnesota: a 9,500 head, cross-ventilated barn with a rotary parlor (A), a 300 head, naturally-ventilated barn and an automatic milking system (B), a 200 head, naturally-ventilated barn and a parabone parlor (C), a 400 head, naturally-ventilated barn and a parallel parlor (D), and a 275 head, pasture-based farm with a parabone parlor (E). Electricity was monitored from July 2018 to December 2019. Multiple electric loads were monitored on the farm side of the electric utility meter at the circuits. Electrical use was evaluated on an electrical load basis (Table 1). Despite barn design and cow numbers on farm, ventilation was the largest user of electricity across farms A, B, and C. Ventilation use ranged from 12% to 36% of total electricity used across all farms. Electricity for lighting ranged from 4 to 19% of total electricity usage. Manure handling ranged from 0.02 to 24% of total electricity usage. Milk cooling (compressors and chillers) ranged from 5 to 34% of total electricity usage. Approximately 14% of the electricity usage on Farm B was for operation of the automatic milking system. The annual mean kWh of electricity per cow was: 965 kWh/cow on Farm A, 1,145 kWh/cow on Farm B, 574 kWh/cow on Farm C, 775 kWh/cow on Farm D, and 400 kWh/cow on Farm E. Mean electricity to produce one kg of milk ranged from 0.04 kWh/kg on Farm C to 0.11 kWh/kg on Farm A. Monthly mean electricity per kg/fat plus protein ranged from 0.64 kWh/kg on Farm C to 1.25 kWh/kg on Farm B.

Table 1 (Abstr. 138). Percent of total electricity used by various loads on each farm

	Farm				
Item	A	B	C	D	E
Mean kWh per month	679,476	30,863	8,725	27,346	7,527
Electricity use, % of total kWh[1]					
Ventilation, %	36.0	24.0	16.0	13.0	12.0
Lights, %	16.0	7.0	12.0	19.0	4.0
Manure handling, %	9.0	2.0	0.02	34.0	1.0
Milk cooling, %	8.0	23.0	17.0	15.0	37.0
Automatic milking system, %	NA	14.0	NA	NA	NA

[1] Includes the highest consuming electrical loads on each farm.

Key Words: dairy, electricity

139 Use of agrivoltaics to shade cows in a pasture dairy system. K. Sharpe*, B. Heins, E. Buchanan, and M. Reese, *West Central Research and Outreach Center*, *Morris, MN*.

Recently, the combined use of solar photovoltaics and agriculture has been increasing and may provide farmers with an alternative means of income while increasing the health of their dairy cows. The objective of this study was to determine the effects of grazing cattle under shade cast by a solar photovoltaic system. The study was conducted at the University of Minnesota West Central Research and Outreach Center's, Morris, MN, organic dairy. Twenty-four crossbred cows were randomly assigned to treatment groups from June to September 2019. The replicated (n = 4) treatment groups of 6 cows each were shade from a 30 kW photovoltaic system in a pasture or no shade on pasture. Behavioral observations and production were evaluated on cows during 4 periods of the summer months. Smaxtec boluses (smaXtec, Graz, Austria) and a sensor (CowManager SensOor, Agis Automatisering BV, Harmelen, the Netherlands) monitored internal body temperature and activity and rumination on all cows. Data were analyzed using PROC MIXED of SAS. Independent variables were the fixed effects of breed, treatment group, coat color, period, and parity. No differences ($P > 0.05$) in fly prevalence, milk production, fat and protein production, BW, body condition score, drinking bouts, hock lesions, or locomotion were found between the treatment groups. Shade cows had more ($P < 0.05$) ear flicks (11.4 ear flicks/30 s) than no shade cows (8.6 ear flicks/30 s) and had dirtier ($P < 0.05$) bellies and lower legs (2.2 and 3.2, respectfully) than no shade cows (1.9 and 2.9, respectfully). During the afternoon, shade cows had lower ($P < 0.05$) respiration rates (66.4 breaths/min) than no shade cows (78.3 breaths/min). From 12:00 to 18:00 h and 18:00 to 00:00 h, shade cows had lower ($P < 0.05$) body temperatures (39.0 and 39.2°C, respectfully) than no shade cows (39.3 and 39.4°C, respectfully). Furthermore, during daylight hours and between milkings, the shade cows had lower ($P < 0.05$) body temperatures (38.9°C) than no shade cows (39.1°C). Incorporating agrivoltaics into a pasture dairy system may increase the health of dairy cows, reduce heat stress, and increase the efficiency of the land.

Key Words: agrivoltaics, dairy, pasture

Ruminant Nutrition: Calves and Heifers

140 Performance and digestibility of dairy calves fed milk replacer with all-milk protein or with 35% of vegetable proteins.

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The effect of replacing 35% of milk protein by vegetable protein in milk replacers (MR; 24:18% CP:EE) to young calves in an intensive rearing system was evaluated. Forty-eight Holstein male calves (41 ± 8 kg of BW; 3–5d old), were blocked by BW and birthdate, and randomly assigned to 4 treatments (Trt): (1) 100% milk protein (MP), (2) 65% MP + 35% micronized soybean meal (MS), (3) 65% MP + 35% wheat gluten (WG), or (4) 65% MP + 35% mixed 50:50 MS and WG (SW), offering MR at 20% of BW, 12.5% of DM, twice daily, weaning at d 60. Water and starter (21% CP, since d 21) were offered ad libitum. Intake and BW were recorded daily and weekly, respectively. Total feces were collected from each calf on d 14–18 (digestibility); blood samples were taken at d 23 and 51 (glucose and insulin). All variables were analyzed using a mixed model. Intake, BW, ADG, feed efficiency (FE), glucose, and insulin were analyzed as repeated measures over week (Wk). There was no Trt effect on MR intake (937 ± 10.9 g DM/d; $P = 0.40$), starter intake (390 ± 31.0 g DM/d; $P = 0.28$), ADG (738 ± 30.0 g/d; $P = 0.95$), final BW (85.9 ± 1.8 kg/d; $P = 0.95$), and FE (635 ± 24.0 g/d; $P = 0.54$). Replacing 35% of MR protein by MS or WG decreased DM and CP digestibility, but this was not observed when 50:50 mix of MS-WG was used, consuming calves on SW more digestible CP than MP and MS. We found a treatment effect on insulin and insulin to glucose, but not on glucose (Table 1). It is possible that WG and SW affected insulin responsiveness, but more information is needed to confirm it. Therefore, replacement up to 35% of MR protein by a 50:50 mix of MS-WG allows to achieve similar digestion and performance than MR with 100% of MP.

Key Words: milk replacer, protein sources, digestibility

141 *Schizochytrium* sp. and lactoferrin improve intestinal health of dairy calves challenged with *Escherichia coli* K99.

A. L. T. Zhu La[1], W. H. Liu[1], L. J. Ren[1], S. T. Gao[1], Z. T. Yu[2], D. P. Bu[1], and L. Ma*[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Department of Animal Sciences, The Ohio State University*, *Columbus, OH*.

Calf diarrhea is a common intestinal disease, and *Escherichia coli* is the main causative agent. The aim of this study was to evaluate *Schizochytrium* sp. (SZ) and lactoferrin (LF) for their ability to protect intestinal health of dairy calves challenged with *Escherichia coli* K99. Forty newborn male Holstein calves (1 d old, 39.9 ± 4.5 kg initial BW) were randomly assigned to 1 of 4 groups (n = 10): 1) Ctrl: control fed whole milk only; 2) LF: fed whole milk supplemented with lactoferrin (1 g/d); 3) SZ: fed whole milk supplemented with *Schizochytrium* sp. (20 g/d); and 4) LF+SZ: fed whole milk supplemented with both lactoferrin (1 g/d) and *Schizochytrium* sp. (20 g/d). The SZ and LF were added to whole milk (protein: fat ratio, 3.64%:4.20%) immediately before feeding and fed daily to each calf from d 2 to 14. At d 7, all the calves were challenged with *Escherichia coli* K99 (1×10^{10} cfu/mL) once orally. During the experiment, milk and starter feed intake and fecal scores were recorded daily, while BW (BW) was weighted at d 1, 7, and 14. Blood was sampled after the afternoon feeding from the jugular vein at d 7 and 14. Statistical analysis was performed using SAS v. 9.4 with SZ, LF, the interaction of SZ and LF, week, and the 2- and 3-way interactions of SZ, LF, and time as fixed effects. Compared with the control, LF lowered fecal score (2.05 vs 2.30, $P < 0.05$) and nitric oxide concentration in serum (1.63 vs 1.99, $P < 0.05$), while SZ increased immunoglobulin G (IgG) concentration (1,357.23 vs 1,341.91, $P < 0.05$), tended to increase the concentration of interleukin-10 (IL-10) (81.99 vs 77.65, $0.05 < P < 0.1$) and to reduce the concentration of tumor necrosis factor-α (TNF-α) (163.15 vs 166.47, $0.05 < P < 0.1$) in serum. The combination of LF and SZ (LF+SZ) tended (2.05 vs 2.30; 0.51 vs 0.60, $0.05 < P < 0.1$) to lower fecal score and serum lipopolysaccharide concentration but lowered blood D-lactic acid concentration in serum (3.31 vs 3.50, $P < 0.05$). These results indicate that SZ could improve the immunity and anti-inflammatory activities, reduce intestinal permeability and pro-inflammatory activities, and decrease fecal scores in dairy calves, while LF could reduce fecal scores and intestinal permeability. A synergistic effect was shown by this study between LF and SZ in decreasing fecal score and intestinal permeability, which can effectively alleviate diarrhea in dairy calves.

Key Words: *Schizochytrium* sp., lactoferrin, dairy calves

142 Effects of inclusion of a high-fat extruded pellet mixed with a conventional pellet in rearing calves.

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Calf rearing systems are moving toward greater milk allowances (≥20% birth weight in L/d), driven by their benefits for later life performance. However, higher milk allowances can lead to a reduction in solid feed intake before, during and even after weaning, resulting in lower energy intake. Therefore, fat inclusion in the starter pellets can increase energy density of the feed, but it might negatively influence rumen development. The objective of this study was to evaluate the effect of supplementing a high-fat extruded pellet mixed with a conventional starter on feed and energy intake, and performance in calves. Forty Holstein bull calves were blocked by serum IgG (2451 ± 109 mg/dL) and date of arrival (2.5 ± 0.5 d of age). Within each block, calves were randomly assigned to 1 of 2 treatments:

Table 1 (Abstr. 140). Effect of replacing milk protein by vegetable protein in milk replacers

	Treatment					*P*-value		
Item	MP	MS	WG	SW	SEM	Trt	Wk	Trt × Wk
DM dig, %	94.8^{a}	91.1^{b}	91.3^{b}	92.6^{ab}	0.85	0.01	—	—
CP dig, %	91.0^{a}	82.8^{b}	82.7^{b}	88.3^{ab}	1.83	<0.01	—	—
Dig CP intake, g/d	196^{b}	193^{b}	203^{ab}	226^{a}	10.0	0.02	—	—
Glucose, mg/L	3.0	2.9	3.1	3.0	0.14	0.65	0.01	0.09
Insulin, µU/mL	5.8^{b}	6.7^{ab}	9.2^{a}	9.2^{a}	1.16	0.03	0.73	0.06
Insulin to glucose, µU/mg	19.3^{a}	22.7^{ab}	29.7^{b}	29.4^{b}	3.29	<0.01	0.95	0.36

a pelleted low fat control starter (LFP; 3.2% fat) or the LFP mixed in a ratio of 90:10 with an extruded feed high in hydrogenated palm free fatty acids (HPFFA; 6.8% fat for the mixture). Calf milk replacer (MR: 24.4% CP, 19.6% CF) was provided twice daily until 42 d of age, followed by a gradual weaning period of 7 d. Starter treatments, straw and water were available ad libitum throughout the study (2 to 112 d of age). Measurements included intake, BW, and blood parameters. Data were analyzed by PROC MIXED in SAS accounting for the fixed effects of treatment, time (week), and their interaction, plus the random effect of block. Measurements over time entered the model as repeated measures. No differences were observed between LFP and HPFFA calves on BW or ADG until 49 d of age. From weaning (d50) until d 112, HPFFA calves had a greater BW ($P < 0.05$) than LFP animals (140.0 vs. 131.5 kg). Moreover, HPFFA calves had the highest starter, straw, calculated metabolizable energy and CP intakes after weaning ($P < 0.05$). No differences were present in blood B-hydroxybutyrate, glucose or IGF-1 concentrations between treatments. In conclusion, the high-fat extruded pellet in at 10% mixed with a conventional starter improved BW, solid feed and energy intake after weaning.

Key Words: calf weaning, fat inclusion, solid feed

143 Effect of dietary energy source replacement in calf milk replacer fed ad libitum on voluntary feed intake and performance in dairy calves. J. Echeverry-Munera*[1,2], L. Leal[2], J. Wilms[2], H. Berends[2], M. A. Steele[1], and J. Martin-Tereso[2], [1]*University of Guelph, Guelph, ON, Canada*, [2]*Trouw Nutrition, Amersfoort, the Netherlands*.

Compared with whole milk, most commercial calf milk replacers (MR) deliver relatively high levels of lactose (42–45 vs. 35% inclusion), low levels of fat (18–20 vs. 30%), and protein levels that are rather comparable (~23%), resulting in lower energy density and energy to protein ratio of the diet. Thus, the study objective was to determine the effect of exchanging lactose by fat in MR on voluntary feed intake and growth performance. Thirty-two Holstein bull calves (2.1 ± 0.16 d of age, 46.4 ± 0.77 kg BW) were assigned to 1 of 16 blocks based on arrival date and serum IgG. Within each block, calves were randomly assigned to 1 of 2 treatments: High lactose MR (HL, 18% fat; n = 16), or high fat MR (HF, 24% fat; n = 16). Lactose was exchanged by fat on a wt/wt basis, resulting in a different energy content. The experiment was divided into 3 phases: Pre-weaning (P1; 0–35 d), weaning (P2; 36–63 d) and post-weaning (P3; 64–84 d). In P1, calves were initially housed in individual pens and fed their respective MR ad libitum through teat buckets and water through plain bucket. After 2 weeks, calves were group-housed (8 calves/ pen; 4 blocks) and fed ad libitum MR, starter feed, chopped wheat straw, and water via automated feeders. During P2, calves were gradually weaned until d-63. Measurements included intakes and BW. Data was analyzed by PROC MIXED in SAS accounting for the fixed effects of treatment, time, and their interaction, as well as the random effect of block. Measurements over time entered the model as repeated measures. Increasing fat content at the expense of lactose decreased MR intake during P1 by 15% (HL = 8.8; HF = 7.6 L/d), whereas total starter intake was not affected by MR composition (HL = 1.8; HF = 1.7 kg/d). Average daily gain (ADG) was higher for HL calves during P1 (0.837 vs. 0.729 g/d); however, no differences were found during P2 or P3. Final BW (d-84) did not differ between treatments. In conclusion, calves fed ad libitum seem to regulate their MR intake based on energy content of the diet, without significant effects on solid feed intake and overall BW.

Key Words: milk replacer, lactose, fat

144 Health and performance of dairy calves supplemented with prebiotics: A systematic review. R. Branco Lopes* and N. Silva-del-Río, *Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA*.

The objective of this study was to systematically review and summarize the current literature on the effect of prebiotic supplementation on performance and health of dairy calves. A review protocol was developed in accordance with PRISMA-P guidelines. The literature search was performed on Oct-2018 and updated on Dec-2019 using 5 electronic databases (CAB Abstracts, PubMed, Science Direct, Scopus and Web of Science). Eligible studies were non- and randomized controlled trials available in English, Portuguese, and Spanish, that evaluated the effect of prebiotic supplementation on dairy calves' performance and health. For randomized trials, the risk of bias was assessed at the outcome (average daily gain; ADG) level, using the Cochrane Collaboration's tool. A total of 2,199 publications were retrieved, and 27 manuscripts (32 trials) met the inclusion criteria. Twenty-one studies were randomized controlled trials; among those assessed for risk of bias, most did not report the randomization method. Forty-eight percent of the studies received financial support or were affiliated with a prebiotic industry. Trials size ranged from 4 to 60 calves/treatment group; none of the studies included sample size calculations. All the studies used HO or HO crossed calves (n = 1458) with age at enrolment ranging from 0 to 162 d (mean ± SD = 14 ± 28). The prebiotic supplementation was mostly via milk replacer (n = 15) or whole milk (n = 7) during 7 to 143 d (52 ± 33). Twenty studies investigated oligosaccharides; mainly fructo- (n = 5) and mannan-oligosaccharides (MOS; n = 5). Among the manuscripts evaluating performance (n = 21), 16 did not observe effect on ADG. Only 5 studies (n = 2 inulin; n = 1 galacto-oligosaccharides; n = 1 MOS; n = 1 polysaccharides) reported a positive effect of prebiotic supplementation on growth. Two of those studies presented a high risk of detection bias; BW was estimated with measuring tape and the personnel was not blinded. Sixty percent of studies evaluating fecal consistency (n = 6/10) did not report positive effect. To allow proper knowledge summary, future research should adhere to guidelines for performance and health measurements.

Key Words: calves, review

145 Impact of supplementary trace mineral source in the diet of prepartum cows on quality of colostrum and passive transfer of immunity in newborn calves. L. Ogilvie*[1], B. Mion[1], J. F. W. Spricigo[1], B. Van Winters[1], B. W. McBride[1], S. J. LeBlanc[2], M. A. Steele[1], and E. S. Ribeiro[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada*.

Our objectives were to evaluate the impact of supplementary trace minerals source, inorganic (ITM; Co, Cu, Mn, Zn sulfates and sodium selenite) or organic (OTM; Co, Cu, Mn, Zn proteinates and Se yeast; Bioplex Sel-Plex, Alltech Inc.), fed to prepartum cows at 100% of recommended levels (0.25, 13.7, 40.0, 22.8, and 0.3 ppm respectively), on quality of colostrum and passive transfer of immunity in newborn calves. Heifers and cows (n = 240) were enrolled at 45 ± 3 d before expected calving date, blocked by parity and BCS, and allocated randomly to ITM or OTM. Automatic feeding gates were used to assign treatments to individual cows. Shortly after calving, cows were milked and the volume of harvested colostrum was measured. Percentage Brix was determined by refractometer and concentration of IgG in colostrum was determined by radial immunodiffusion. Newborns were fed 3L of colostrum (%Brix >22) in the first 6h of life, and additional 3L 6h later. Concentration of total protein and IgG in serum was determined 24h after colostrum feeding using refractometry and radial immunodiffusion, respectively. Continuous data were analyzed by ANOVA and binary data were analyzed by logistic regression using the PROC GLIMMIX of SAS. Models included the effects of treatment, parity, season, and their interactions. Analyses of data from 182 cows indicated no differences in harvested volume (SQRT scale: ITM = 2.1 vs. OTM = 2.2 ± 0.06 L; $P = 0.21$), Brix% (ITM = 24.6 vs. OTM = 24.6 ± 0.4%; $P = 0.94$), and IgG concentration (SQRT scale: ITM = 8.6

vs. OTM = 9.0 ± 0.2 mg/mL; $P = 0.20$) in colostrum of ITM and OTM cows. Total secretion of IgG in colostrum (volume*concentration) was greater in OTM than in ITM (SQRT scale: ITM = 17.4 vs. OTM = 19.4 ± 0.6 g; $P = 0.03$). No differences were observed in birth BW (ITM = 42.1 vs. OTM = 42.1 ± 0.5 kg; $P = 0.62$), concentration of total serum protein (ITM = 6.3 vs. OTM = 6.3 ± 0.1 g/dL; $P = 0.92$), and serum IgG (ITM = 30.2 vs. OTM = 31.4 ± 1.6 mg/mL; $P = 0.59$) between calves born to ITM and OTM cows. Our results indicate that replacement of ITM by OTM in the prepartum diet affected the total amount of IgG secreted in colostrum but did not impact the passive transfer of immunity in newborn calves

Key Words: trace minerals, colostrum, immunoglobulin G

146 The effect of pre-, pro-, and symbiotic supplementation in milk to pre-weaned Holstein heifers on body weight gain and health outcomes.

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The objective of this trial was to evaluate the effectiveness of pro, pre and symbiotic supplementation on average daily weight gain (ADG), diarrhea and odds of pneumonia treatment with an antibiotic in pre-weaned dairy heifer calves. A total of 1,801 healthy Holstein heifer calves from a large California dairy were enrolled at 4 – 12 h of age until weaning at approximately 60 d of age. Calves were block-randomized based on birth order on a repeating random order of all possible orderings of the 4 treatments: 1) control, 2) enzymatically hydrolyzed yeast product (pre), 3) *Bacillus subtilis* (pro), 4) combination of both products (symbiotic), which were administered in milk twice daily from enrollment until weaning. Serum total protein at enrollment and BW at 7, 42 and 56 d of age were measured. Fecal consistency was assessed daily on a scoring system of 1 −3, for the entire pre-weaned period. Linear regression showed that symbiotic treated calves had 20 g increased ADG compared with control calves for overall BW gain (7 – 56 d) ($P = 0.042$), controlled by calf location in farm, month of enrollment and pneumonia incidence. For late BW gain (42 – 56 d), prebiotic treated calves had 84 g increased ADG ($P = 0.007$) and symbiotic treated calves had 79 g increased ADG ($P = 0.012$) compared with control calves, controlled by month of enrollment. Linear regression showed no difference in the length of the first diarrhea episode per calf by treatment. Cox regression showed no difference in hazard of diarrhea compared with control calves. Logistic regression showed no difference in the odds of calves experiencing pneumonia. Although no effects were seen on diarrhea length or pneumonia incidence, superior BW gain in the late pre-weaned period may increase health outcomes of calves across the weaning process.

Key Words: calf, probiotic, prebiotic

Ruminant Nutrition: Gut Physiology, Fermentation, and Digestion

147 Effects of probiotics on in vitro digestibility, rumen fermentation, methane and gas production from a corn silage-based TMR. A. Oyebade*, K. G. Arriola, H. Sultana, S. Lee, E. Duvalsaint, F. Amaro, I. Fernandez Marenchino, C. Nino De Guzman, L. Marroquin Pacheco, L. Ghedin Ghizzi, M. Reboucas Pupo, M. Agarussi, L. Ferraretto, A. Adesogan, D. Vyas, *University of Florida*, *Gainesville, FL*.

The study objective was to evaluate effect of 2 probiotic supplements on in vitro nutrient digestibility, methane (CH_4) and volatile fatty acid (VFA) production using in vitro batch fermentation assay. Three ruminally-cannulated cows were used in a 3×3 Latin Square design and fed either basal corn silage-based TMR without probiotics (Control; CON), the basal diet top-dressed with mixture of *Lactobacillus animalis* and *Propionibacterium freudenreichii* at 3×10^9 cfu/day (BOV) or the basal diet top-dressed with BOV and mixture of *Bacillus subtilis* and *B. licheniformis* at 11.8×10^9 cfu/day (BOVC). Each experimental period was 28-d, and rumen fluid was collected from each cow, 3 h after feeding on d 22, 24, 27, and 28 of the experimental period for 4 runs of in vitro batch fermentation assay per period. Rumen fluid collected from each donor cow corresponded to the specific dietary treatment the cow was receiving. Additionally, 2 more treatments (BOV+ and BOVC+) were included in the assay by adding extra dose (1.62 mg/mL) of the 2 probiotic supplements (BOV and BOVC) to the vials containing their respective rumen fluids. A corn silage-based TMR (1mm; 0.5 g) used as substrate was incubated in serum vials with respective rumen fluid buffered with Van soest medium and reducing agent at 39°C. Data were analyzed using GLIMMIX procedure of SAS and differences were considered significant at $P \leq 0.05$. Treatments were used as fixed effects while cow and run were considered random factors. No treatment effects were observed on digestibility parameters. The BOV and BOV+ inocula decreased molar proportion of butyrate (13.8 and 13.6%), compared with CON (14.3%), while BOVC+ tended ($P = 0.07$) to decreased molar proportion of butyrate. BOV+ lowered gas production (143.9 vs 162.8 mL/g DMD), and tended to reduce CH_4 production (9.07 vs 11.6 mg/g DMD), compared with CON. BOVC reduced ruminal acetate (50.2 vs 51.1%) and tended to reduce isovalerate ($P = 0.07$) molar proportion. In conclusion, probiotic supplementation did not change in vitro nutrient digestibility; however, it tended to lower CH_4 production.

Key Words: methane, probiotics, rumen fermentation

148 *Saccharomyces cerevisiae* fermentation products reduce effects of subacute ruminal acidosis on CowPi-predicted functionalities of rumen microbiota in lactating dairy cows. J. Guo*[1], Z. Zhang[3], I. Yoon[2], M. Zhou[3], L. Guan[3], E. Khafipour[2], and J. Plaizier[1], [1]*University of Manitoba*, *Winnipeg, MB, Canada*, [2]*Diamond V*, *Cedar Rapids, IA*, [3]*University of Alberta*, *Edmonton, AB, Canada*.

Cows were randomly assigned to 4 treatments (n = 8) that received a TMR (34.9% NDF, 18.6% starch, DM basis) supplemented with 140 g/d of ground corn (Control), 126 g/d of ground corn and 14 g/d of XPC (XPC, Diamond V Original XPC, Cedar Rapids, IA), 121 g/d of ground corn and 19 g/d of NutriTek (NTL, NutriTek, Diamond V), or 102 g/d of ground corn and 38 g/d of NutriTek (NTH) from 4 wk before until 12 wk after calving. Subacute ruminal acidosis(SARA) challenges were conducted during wk 5 (SARA1) and wk 8 (SARA2) after calving by replacing 20% of the TMR with pellets containing 50% barley and 50% wheat. Rumen samples were collected weekly from wk 3 until wk 10 after calving. DNA was extracted, Illumina sequenced at the V1–V2 regions of the 16S rRNA gene, and analyzed by QIIME2. The OTU counts and sequence tables were constructed in the galaxy implementation of CowPI to predict the Kegg ortholog abundances at the pathway-level. Out of 205 functions, 165 were changed by SARA. The relative abundance of arachidonic acid metabolism increased, but this was reduced by NTH ($P = 0.03$) during SARA2. The abundance of glycosaminoglycan degradation was lower during NTL than during Control (0.038% vs 0.044%, $P = 0.01$. The nod-like receptor signaling pathway decreased more during SARA2 than SARA1 ($P < 0.01$), and NTH increased its relative abundance compared with Control (0.065% vs 0.063%, $P = 0.05$). Retinol metabolism tended to increase more during SARA2 than SARA1 ($P = 0.08$), and NTH decreased its abundance compared with Control (0.0031% vs 0.0035%, $P = 0.05$). Lipopolysaccharide (LPS) biosynthesis and LPS biosynthesis proteins increased during SARA challenges ($P < 0.01$), which agrees with increases in rumen free LPS observed in a parallel study. SARA challenges altered predicted rumen functionalities related to inflammation, and NutriTek attenuated these changes.

Key Words: *Saccharomyces cerevisiae* fermentation product, SARA, rumen microbiota

149 Influence of hydrolyzed soybean meal on glycogen production by mixed ruminal microbes. M. B. Hall* and M. L. Sullivan, *U.S. Dairy Forage Research Center, USDA-ARS*, *Madison, WI*.

Increasing the supply of degradable protein can alter products formed by mixed ruminal microbes, decreasing glycogen and increasing microbial nitrogen. The objective of this study was to evaluate the effect of supplementing supernatant (SUP) from enzymatically hydrolyzed soybean meal (SBM) on glycogen production by mixed ruminal microbes. SBM (20% solids with distilled water; dH2O) with (+e) or without (-e) ENZECO Protease BL was incubated with continuous mixing for 1 h at 60°C. SUP was isolated by centrifugation at 15,000 x g for 10 min at 5°C. SUP N mg/mL were -e: 5.87 and +e: 13.31. Replicate in vitro fermentations (3) were performed with Goering-Van Soest medium without tryptone. Each tube contained 20 mL medium, 1 mL reducing solution, 79.5 mg glucose in 0.5 mL, 1 mL of tryptone solution or 0.2, 0.4, or 0.6 mL of SUP brought to 1 mL with dH2O, and 5 mL of strained rumen fluid. Increasing SUP delivered N mg/tube of 1.17, 2.35, and 3.52 for -e, 2.66, 5.32, and 7.99 for +e, and 2.66 or 5.32 for tryptone. -e gave insight to the impact of SUP carbohydrate addition from SBM. Tubes harvested at 3 h of fermentation were analyzed for microbial glycogen. Data were analyzed as a randomized complete block design with run as a random variable. Gel electrophoresis showed the pattern of proteins in -e was similar to that in solubilized SBM; for +e, higher molecular weight proteins present in SBM were reduced and more lower molecular weight proteins were apparent. Glycogen amount was greater with -e (15.2 mg) than +e (13.6 mg; Effects: protease, $P < 0.01$; supernatant dose, $P = 0.76$; interaction, $P = 0.20$; SED 0.68) with a tendency for a quadratic effect of SUP dose ($P = 0.08$). Comparison of +e and tryptone at the 2 common levels of N gave glycogen values of 13.8 mg for +e and 11.6 mg for tryptone ($P < 0.01$) with no effects of N dose ($P = 0.17$) or the interaction ($P = 0.97$; SED = 0.56). Lower glycogen values for +e vs. -e and for tryptone vs. +e indicate reduced storage of glycogen with +e and tryptone, describing potentially more efficient fermentations, ostensibly due to relatively greater amounts of more immediately available degradable protein and peptides.

Key Words: fermentation, protein, glycogen

150 Effect of autochthonous Nepalese plants on ruminal methane production in vitro. R. Dhakal[1], E. Vargas-Bello-Pérez*[1], M. González-Ronquillo[2], and H. H. Hansen[1], [1]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen*, *Frederiksberg C, Denmark*, [2]*Facultad de Medicina Veterinaria y Zootecnia, Instituto Literario 100, Universidad Autónoma del Estado de México*, *Toluca, Estado de México, México*.

The objective of this study was to determine the use of dried fruits from commonly used Nepalese plants on rumen fermentation in vitro. Two plants and a plant product were tested in 3 concentrations: the fruit of *Terminalia chebula* (HA), *Terminila belliricia* (BA) and *Triphala Churna*

(TC), a commercial mixture with 3 equal parts (DM basis) of *Phyllanthus emblica*, *Terminila belliricia*, and *Terminalia chebula*. These plants/product were tested pure and at 20% and 40% of the total sample dry matter in maize silage (MS). Additionally, pure MS was used as a control. These 10 treatments were tested during 2, 48-h fermentations with quadruplicate samples. Rumen fluid was collected from 2 fasting cannulated heifers. Total gas production (TGP: mL at standard temperature and pressure (STP)/g DM), methane production (MP), and volatile fatty acids contents (VFA) were measured or calculated from measurements. Data were analyzed with linear mixed models using R version (3.5.1) with each treatment as a main effect and run as a random variable. Total gas production and MP were less ($P < 0.05$) than MS (186 mL gas/g DM with 10% methane) for pure samples, but not significantly different from MS for 20 and 40% plants/product mixtures with a reduction in TGP between 5 and 15 and 8 to 16% for methane. Neither total VFA production (mmol/L) nor proportions of propionic acid and acetate (mol/100mol) were significantly different between MS and plant/product and MS mixtures ($P > 0.05$). The reduction in MP and similarities between total VFA, TGP, and VFA profiles for the plants/product mixtures and pure MS suggest that these can be used with MS at the given inclusion rates. The decrease in methane represents a heat energy value of between 2.3 and 5.5 MJ/kg of DM, which the animal can theoretically use. Therefore, these plants/product may increase productivity while decreasing enteric methane emission.

Key Words: digestibility, fermentation, methane

151 Effects of feeding a yeast culture product on performance, blood biomarkers, rumen fermentation, and rumen bacteria species in peripartal dairy cows. N. A. Carpinelli*[1], J. Halfen[1,2], S. D. L. Ramirez[1,3], E. Trevisi[4], J. D. Chapman[5], E. D. Sharman[5], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University*, *Brookings, SD*, [2]*Núcleo de Ensino, Pesquisa e Extensão em Pecuaria (NUPEEC), Universidade Federal de Pelotas*, *Pelotas, RS, Brazil*, [3]*Universidad de la Salle*, *Bogotá, DC, Colombia*, [4]*Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Science*, *Italy*, [5]*Phibro Animal Health Corporation*, *Teaneck, NJ*.

Feeding yeast culture fermentation products has been associated with improved feed intake and milk yield in transition dairy cows. The objective of this study was to evaluate the effects of a commercial yeast culture product (Cellerate Culture Classic HD (YC); Phibro Animal Health, Teaneck, NJ) on performance, blood biomarkers, and rumen fermentation and bacterial population in dairy cows during the transition period until 50 DIM. Forty Holstein dairy cows were enrolled in a randomized complete block design from −30 to 50 DIM and blocked according to expected calving day, parity, previous lactation milk yield, and genetic merit. At −30 DIM cows were assigned to either a basal diet plus 114 g/d of ground corn (CON; n = 20) or basal diet plus 100 g/d of ground corn and 14 g/d YC (n = 20), fed as a top-dress. Cows received the same close-up diet from −30 until calving (0.63 Mcal/kg DM and 12.3% CP) and lactation diet from calving to 50 DIM (0.73 Mcal/kg DM and 15.6% CP). Blood samples were collected at −30, −15, 7, 14, and 30 DIM to evaluate biomarkers of muscle body mass, metabolism, and oxidative stress. Rumen fluid (~50 mL) was sampled via esophageal tubing on −59, −30, 5, and 30 DIM for analysis of ammonia, VFA, and bacterial populations via qPCR amplification. Data were analyzed using the MIXED procedure of SAS. There was a trend ($P \leq 0.13$) for increased milk yield (+2.5 kg/d), ECM (+3.2 kg/d), and ECM/DMI (+0.13) for cows fed YC than CON. A trend ($P = 0.10$) for lower NEFA in YC than CON cows was observed. A trend ($P = 0.08$) for a Diet × Time was observed for butyrate percentage, where lower ($P = 0.03$) butyrate was observed in YC than CON at 5 DIM. Rumen bacteria associated with improved feed efficiency such as *Megasphaera elsdenii* was greater ($P = 0.05$) in YC cows compared with CON while *Prevotella albensis* was greater ($P = 0.01$) in YC compared with CON cows at 30 DIM. These results suggest that feeding YC to transition dairy cows can affect rumen fermentation by altering rumen bacteria populations, which helps explain the observed improvements in milk yield and milk yield efficiency.

Key Words: transition cow, yeast culture, rumen bacteria

152 Yeast culture supplementation effects on lactation performance, rumen fermentation characteristics, and abundance of major species of ruminal bacteria in mid-lactation Holstein dairy cows. J. Halfen*[1,2], N. Carpinelli[1], J. Chapman[3], E. D. Sharman[3], J. L. Anderson[1], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University*, *Brookings, SD*, [2]*Núcleo de Ensino, Pesquisa e Extensão em Pecuária (NUPEEC), Departamento de Clínica Veterinaria, Universidade Federal de Pelotas*, *Plotas, RS, Brazil*, [3]*Phibro Animal Health Corporation*, *Teaneck, NJ*.

Improvements in milk yield and efficiency have been observed when feeding yeast culture products to dairy cows, and this has been partially attributed to alterations in rumen fermentation. Therefore, this study aimed to evaluate the effects of feeding yeast culture [Cellerate Culture Classic HD (YC); Phibro Animal Health, Teaneck, NJ] on lactation performance, rumen fermentation characteristics, and rumen bacterial populations in lactating dairy cows. Forty mid-lactation Holstein dairy cows (121 ± 43 DIM; mean ± SD) were used in a randomized complete block design consisting of 7 d adaptation period and 60 d treatment period. Cows were blocked by parity, DIM, and previous lactation milk yield and assigned to a basal TMR plus 114 g/d of ground corn CON (n = 20) or basal TMR plus 100 g/d of ground corn and 14 g/d YC (n = 20). Blood and rumen fluid samples were collected at 0, 30, and 60 d. Rumen fluid was analyzed for ammonia-N, VFA concentrations, and relative abundance of bacterial populations via qPCR amplification. Data were analyzed using the MIXED procedure of SAS. Dry matter intake tended ($P = 0.07$) to be 1.4 kg/d lower in YC than CON cows, while milk yield was not affected ($P = 0.43$) by diet. Consequently, a trend ($P = 0.07$) was observed for greater milk efficiency in YC than CON cows (1.46 vs. 1.34 milk/DMI). The trend ($P = 0.11$) for a Diet × Time interaction in rumen pH resulted in greater rumen pH in YC than CON cows at 60 d. Greater ($P = 0.02$) propionate was observed in YC than CON (26.9 vs 25.4%). A trend ($P = 0.07$) for lower acetate was observed in YC than CON (60.2 vs 61.2%). There was lower ($P = 0.04$) acetate:propionate ratio in YC than CON. *Prevotella albensis*, associated with improved feed efficiency, was greater ($P = 0.05$) in YC than CON. *Ruminobacter amylophilus*, related to rumen acidosis, had a Diet × Time effect ($P < 0.01$) with lower ($P < 0.01$) abundance in YC than CON at 30 d. These results suggest feeding YC may help maintain milk production during transient reductions in DMI, by modulating rumen microbiota and fermentation patterns.

Key Words: yeast culture, rumen bacteria, dry matter intake

418 The effects of neomycin inclusion in milk replacers on the health, growth, and performance of male Holstein calves. L. N. Buss*[1], T. T. Yohe[1], L. R. Cangiano[1], A. J. Keunen[2], D. L. Renaud[1], L. L. Guan[3], and M. A. Steele[1], [1]*University of Guelph, Guelph, ON, Canada*, [2]*Mapleview Agri. Ltd., Mapleton, ON, Canada*, [3]*University of Alberta, Edmonton, ON, Canada*.

The prophylactic use of oral antimicrobials is common practice in calf rearing, however, the impacts on gut health are largely unknown. The objective of this study was to investigate the effects of oral antibiotic (neomycin) on calf health, performance, and gastrointestinal development. One hundred fifty calves, less than a week old, were blocked by BW and randomly assigned to 1 of 3 treatments (n = 50 per treatment): control (CON: non-medicated milk replacer; MR), short-term antibiotic (ST: neomycin mixed in MR at 20mg/kg BW from d1–14), or long-term antibiotic (LT: neomycin in MR at 20mg/kg BW from d1–28). Fecal dry matter and BW were measured weekly, whereas daily fecal scores (FS) were assigned for the first 28 d. Data were analyzed using the mixed procedure of SAS. Fixed effects included treatment, period, day, and their interactions, and the random effect of calf within treatment. Statistical

significance was considered at $P \leq 0.05$. A treatment effect ($P = 0.01$) was observed for FS, where CON calves experienced higher average FS (0.640 ± 0.031) compared with ST (0.530 ± 0.034) and LT calves (0.409 ± 0.034). CON calves also experienced longer bouts ($P < 0.01$) of diarrhea (5.44 ± 0.35 d) compared with ST (3.78 ± 0.39 d) and LT calves (3.82 ± 0.38 d). At d7, significant differences were observed in fecal dry matter ($P < 0.01$), where CON calves experienced lower fecal dry matter (0.130 ± 0.012%) than ST (0.200 ± 0.013%) or LT (0.170 ± 0.013%) calves. No significant differences in average daily gain ($P = 0.475$; SEM = 0.0546) were observed. On d 28, a subset of 36 calves (n = 12 per treatment) were euthanized and dissected to assess gastrointestinal development. Histomorphometric measurements were characterized in the distal jejunum, ileum, and colon. Calves in the LT group had heavier (27.50 ± 2.58 g) duodenums ($P = 0.04$) and deeper (515.58 ± 6.31 μm) distal jejunum crypt depths ($P < 0.001$). The results of this study suggest that while neomycin may affect intestinal morphology and decrease the incidence and severity of diarrhea, it has no effect on growth performance, which questions the efficacy of neomycin inclusion in milk replacer.

Key Words: antimicrobial, gut development, histology

Breeding and Genetics

153 Assessing the use of public weather station data to investigate the effects of heat stress on milk production in Canadian Holstein cattle. I. Campos*, C. Baes, A. Canovas, and F. Schenkel, *University of Guelph, Guelph, ON, Canada.*

The objectives of this study were to identify the temperature-humidity index (THI) thresholds where heat load starts to affect production traits (milk, fat, and protein yield) in the first 3 lactations of Holstein cows, and to assess potential differences in heat stress in Quebec and Ontario. A total of 2.1 million test-day records from 167,620 Ontario cows and 3.1 million records from 225,104 Quebec cows spanning an 11-yr period (2008 – 2018) were used. The climatic data consisted of hourly measurements of ambient temperature and relative humidity. In total 58 weather stations were located within a maximum distance of 20km from each herd. A k-means cluster analysis was performed within each THI >75 to exclude herds that may have some type of cooling system. A linear model was fitted to adjust the phenotypes, which were then plotted against the THI levels for each parity. To identify the THI threshold at which milk production starts to decline, a segmented polynomial was used to describe the shape of the curve of the relationship between production and THI values. During the summer, the average THI was 69 and 68 in Ontario and Quebec, respectively. Two heat stress thresholds were identified for milk yield and protein yield, one being associated with a low decline in the yield and the other one associated with sharper decline. For both provinces, the second threshold was reached at about THI = 79 for both milk and protein yields, while the first threshold was reached at about a THI = 64 – 72 for milk yield and THI = 57 – 64 for protein yield, depending on the province and parity. The expected reduction in milk yield after a THI ~79 was −0.40 kg/d and −0.19kg/d in Ontario and Quebec, respectively. The same features for protein yield were −16.2 g/d and −9.7 g/d. For fat yield, the expected reduction in yield after a THI ~57 was −3.3g/d and −2.6g/d for Ontario and Quebec, respectively. Therefore, the estimated rate decline differed between the 2 provinces, which may be related to the higher percentage of tie stall barns in Quebec (92%) compared with Ontario (<70%).

Key Words: dairy cow, heat stress, temperature-humidity index

154 Estimation of genetic parameters for stayability in organic Holsteins. L. C. Hardie*[1], B. J. Heins[2], and C. D. Dechow[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*University of Minnesota, West Central and Outreach Center, Morris, MN.*

The objective of this study was to estimate genetic parameters for stayability of organic Holsteins and determine genetic relationships with other traits. Stayability (1 = remained in herd to a given parity, 0 = left) was generated for parity 1 through 5 for cows from 16 USDA certified organic farms. Animals with a Holstein sire and no other breed for 3 generations were included. All models included fixed herd-year-season of birth and pedigree completeness and the random effect of animal. Heritabilities for parity 1 (n = 27,630 with 66% staying in the herd from birth to parity 1), 2 (82% staying from parity 1 to 2; n = 12,766) and 3 (78% staying from parity 2 to 3; n = 6,978) were estimated using univariate threshold models. Genetic correlations among them were estimated with bivariate linear models. An across-parity analysis used records (n = 101,201 from 27,630 animals) for individual parities up to 5; the fixed effect of parity and random effect of permanent environment were added. Approximate genetic correlations between stayability and nationally evaluated traits were extrapolated from PTA correlations for bulls with accuracy of PTA stayability greater than 0.35. Heritability estimates for stayability to first, second, and third parities were 0.10 ± 0.01, 0.07 ± 0.02, and 0.07 ± 0.03, respectively. The across-parity heritability estimate was 0.12 ± 0.01 and repeatability was 0.61 ± 0.004. The genetic correlations for stayability to parity 1 and 2, 1 and 3, and 2 and 3 were 0.80 ± 0.08, 0.60 ± 0.22, and 0.81 ± 0.19, respectively. Genetic correlations between stayability and most key traits were positive, but less so for heifers (Table 1). In conclusion, there is a genetic component to stayability that remains consistent across parities such that it can serve to mitigate selection bias in genetic analyses with sparse recording of production traits.

Table 1 (Abstr. 154). Approximate genetic correlations with stayability

Trait	All parities	To parity 1	Parity 1 to 2
Productive life	0.54*	0.33*	0.73*
Livability	0.45*	0.44*	0.64*
Milk yield	0.29*	0.06	0.26*
Daughter pregnancy rate	0.12*	−0.10	0.30*
Heifer conception rate	0.16*	0.12	0.17
Early first calving	0.21*	−0.23*	0.42*

*Differs from 0, $P < 0.05$.

Key Words: organic, herd-life, heritability

155 Genomic evaluation of heifer livability. M. Neupane*, C. P. Van Tassell, and P. M. VanRaden, *Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA, Beltsville, MD.*

Differences in breeds and sire lines suggest the presence of a genetic component for heifer livability (HLIV). Genomic evaluation of this trait can increase profitability and improve animal health and welfare. Evaluations for HLIV were examined from 3,360,847 calf data records for heifers of all breeds born from the year 2009–2016. Data were obtained from the national cooperator database maintained by the Council on Dairy Cattle Breeding, USA. The total number of deaths reported was 134,804 (4.01%), which includes the herds with death loss between 1 and 25%. Total death rate from >2 d of age until the heifer left the herd or until a maximum of 18 mo of age was evaluated, but records were not included until 3 years after the birthdate so that live status of contemporaries could be confirmed by a calving date. Deaths observed until 2 d after birth were included in stillbirth rather than HLIV. The scale used for analysis of HLIV was 0 (died) or 100 (live) and the heritability estimate was 0.4% based on sire model REML. Genomic predicted transmitting abilities (GPTA) for Holstein range from −1.5% to +1.5% with a standard deviation of 0.5% and GPTAs for Jersey range from −0.8% to +0.8% with SD of 0.2% compared with means of about 4% death loss. Reliabilities of genomic predictions for young animals averaged 46% for Holsteins and 30% for Jerseys while corresponding traditional parent average reliabilities averaged 16% and 12%. Correlations of HLIV were 0.44 with productive life, 0.34 to 0.36 with yield traits, 0.37 with calving trait dollars, and 0.36 with early first calving on proven Holstein bulls. The HLIV trait had a favorable genetic trend in recent years, likely because of selection for the correlated traits. The trait HLIV could get 1% of emphasis on net merit index making economic progress of $0.05 million per year. By encouraging more recordings on calf mortality, the reliabilities of evaluations can increase significantly.

Key Words: heifer livability, genomic evaluation, animal welfare

156 Genomic evaluation for abortions and twinning in dairy cattle. N. Vukasinovic*, D. Gonzalez-Pena, J. Brooker, C. Przybyla, and S. DeNise, *Zoetis, Kalamazoo, MI.*

Abortions and twinning are undesirable reproductive outcomes in dairy herds. Both traits are believed to be largely caused by environmental factors. However, studies have shown that there is a substantial genetic component to these conditions. The objective of this study was to develop genetic and genomic evaluation for abortions and twinning in Holstein cattle using single-step genomic BLUP (gBLUP) methodology. Production, reproduction, and pedigree data recorded on farms using herd management software were available for this study. Abortions (ABRT) were defined as a recorded abortion event between 42 and 260 d after the successful breeding. Twinning (TWIN) was defined as a pregnancy resulting in birth or abortion of twin calves (alive or dead). The traits were analyzed using the univariate threshold animal model with repeated observations. The model included the fixed effect of parity (1–5) and the random effects of animal, herd x year x season of calving, and permanent environment. In addition, the model for ABRT included the fixed effect of the breed composition of the embryo (purebred vs. crossbred), the random effect of service sire, and the covariates of milk yield and days open. The data consisted of 3,848,620 and 5,338,162 records with an overall incidence of 11.0% and 3.0% for ABRT and TWIN, respectively. Genotypes were available on 978,368 animals. The estimated heritabilities were 0.077 and 0.089 for ABRT and TWIN, respectively. Predicted transmitting abilities (PTA) were expressed in percent points as deviations from the average estimated probability of a disorder in the base population, which was defined as all animals with phenotypes born in 2015. The PTA ranged from −8.72 to 12.92 and from −6.98 to 20.39 for ABRT and TWIN, respectively, with higher values representing higher risk of having a disorder. The reliabilities of PTA for young genotyped animals without own phenotypes were on average 0.36 and 0.39 for ABRT and TWIN, respectively. Genetic and genomic selection of animals less prone to abortions and twinning could be a useful tool for improving reproductive performance in dairy herds.

Key Words: abortion, twinning, genomic evaluation

157 A feasibility study to implement genetic and genomic evaluations for twinning in Holstein cattle. A. Sewalem*, M. McClure, and K. Olson, *ABS Global*, *DeForest, WI.*

Twin births are detrimental for dairy producers due to their association with several unfavorable effects, including increased reproductive disorder, culling risk, abortions, and calving interval. The aims of this study were to (A) assess the incidence of twinning and evaluate its risk factors; (B) examine this trait's association with other traits of economic importance; (C) to develop genetic and genomic evaluations for twinning. The data included Holstein calving records from 2000 to 2019, comprising 1,804 herds with 14M calving records from 25,868 AI sires. The 2018 overall twinning rate was 4.2% which represented a 9.94% increase from 2010. Twinning rate increased with parity with rates of 1.16%, 4.73%, and 7.86% for parities 1, 2, and 3+, respectively, and AI sires' overall twinning rate ranged from 0 to 13.89%. A genetic evaluation of twinning was carried out using a single-trait linear sire model which included the fixed effects of parity and herd-year-season with random effects of sire and the residual. The sire evaluation results were expressed as an estimated transmitting ability (ETA) representing the expected twinning rate differences among daughters with the average ETA set to 100 and std of 5. The ETAs had a normal distribution ranging from 75 to 115. Traditional evaluation results for twinning were de-regressed and used as pseudo-phenotypes to predict the direct genomic values for animals with 43K SNP genotypes. No markers with large effects were observed. Twinning had statistically significant but low correlations with numerous production and fertility traits which indicate that a direct twinning breeding value is needed to make any significant impact on the trait. In conclusion, this study showed large variation in the predicted twinning breeding values indicating that the implementation of genetic and genomic evaluation for twining will help producers make breeding decisions to reduce their herd's economic risk from twin births.

Key Words: twinning, genetic and genomic evaluations

158 Identification and validation of candidate genes for heat tolerance in Australian Holstein dairy cattle. E. K. Cheruiyot*[1,2], M. Haile-Mariam[1], B. G. Cocks[1,2], I. MacLeod[1], and J. E. Pryce[1,2], [1]*Agriculture Victoria Research, Department of Jobs, Precincts and Regions*, *Melbourne, Australia*, [2]*School of Applied Systems Biology, La Trobe University*, *Melbourne, Australia*.

Heat tolerance is the ability of an animal to maintain production and reproduction levels under hot conditions and is now a trait of economic relevance in dairy systems worldwide. Understanding the genetic basis for heat tolerance is an important part of the strategy to breed for cattle adapted to warmer environments. We sought to identify and validate candidate genes involved in heat tolerance in Australian Holstein cattle using 50K SNP chip and imputed whole-genome sequences (WGS) in 2 data sets: cows (n = 20,623) and bulls (n = 1,622). The WGS was imputed using Run 7 of the 1000 Bull Genome Project based on the ARS-UCD1.2 reference genome. After quality checks, approximately 45K and 15 million SNPs remained for analysis from the 50K chip and the WGS, respectively. The heat tolerance phenotypes that were used for this study were derived from test-day milk, protein and fat yield data of cows that calved between 2003 to 2017 and represent the response of cows to heat stress. For the 3 milk traits, the slope which measures change in milk yield due to variability in temperature-humidity index from reaction norm models calculated for individual animals after accounting for known fixed effect was used as phenotype in association analysis using GCTA software. We used cows for discovery and bulls that were not sires of the cows for validation. Our results point to the polygenic nature of heat tolerance, with no variants surpassing a significance threshold of $P < 1.0 \times 10^{-6}$ for 50K SNP data. While we detected some associations when the threshold was set at $P < 1.0 \times 10^{-3}$, there were high false discovery rates. In contrast, we detected multiple variants with $P < 1.0 \times 10^{-6}$ across the genome using WGS, suggesting greater power compared with 50K SNP data. Across our cow and bull validation set, we confirmed several candidate genes, including some that have been reported previously. Our results are preliminary and could be useful to enhance the reliability of heat tolerance genomic breeding values of Australian Holstein cattle which is currently about 38%. This will be investigated in a further study.

Key Words: heat tolerance, dairy cattle, whole-genome sequences (WGS)

Dairy Foods: Dairy Products

159 Rheological, texture, structural, and functional properties of Greek-style yogurt fortified with cheese whey-spent coffee ground powder. J. Osorio-Arias[1], A. Pérez-Martínez[2], O. Vega-Castro[2], and S. Martinez-Monteagudo*[3], [1]*Faculty of Pharmaceutical and Food Science, Medellin, Colombia*, [2]*Aoxlab S.A.A, Medellin, Colombia*, [3]*Dairy and Food Science Department, South Dakota State University, South Dakota, Brookings, SD.*

The present study evaluated the feasibility of the fortification of Greek-style yogurt with a newly developed ingredient consisting of cheese whey-spend coffee ground (CW-SCG) powder. The yogurts were analyzed using a battery of tests, including whey production, water holding capacity, firmness, rheological properties, protein content, available lysine, and antioxidant activity. The milk base was fortified to 15% dry matter with different skim milk powder to CW-SCG ratios (100/0, 75/25, 25/75, and 0/100 wt./wt.). The addition of CW-SCG up to 75% did not significantly change the acidification curve when comparing with the control sample ($P > 0.05$), reaching the target pH 4.7 after 270–300 min. The available lysine decreased with the addition of CW-SCG, yielding values of 78.55 ± 1.56, 28.89 ± 2.45, 23.61 ± 4.42, 20.03 ± 2.71 mg per 100 g for 0-, 25-, 75-, and 100-CW-SCG, respectively. The highest value of whey production was obtained in those samples fortified with 100-CW-SCG (6.33 ± 0.35%), followed by 75- and 25-CW-SCG (5.17 ± 0.99 and 3.01 ± 0.81%, respectively). The antioxidant capacity increased proportionally to the added CW-SCG powder, yielding values of 68.41 ± 2.78, 80.71 ± 2.54, 100.51 ± 3.44, and 120.21 ± 3.18 μmol TEs per 100 g for 0-, 25-, 75-, and 100-CW-SCG, respectively. Fortification of the yogurt with CW-SCG decreased the water holding capacity by 13 to 25%, depending on the level of fortification. Similarly, the addition of CW-SCG significantly decreased the hardness of the yogurts from 76.57 ± 1.18 to 30.27 ± 1.73 N s ($P < 0.05$). Additionally, fortified yogurts with CW-SCG yielded a product with less shear-thinning behavior as compared with control. The scanning electron images of the fortified yogurts revealed the incorporation of spent coffee ground particles within the protein network. Polyphenol-Protein associations may explain the texture and rheological behavior of the yogurts. The fortification of the yogurt up to 25% of CW-SCG yielded comparable properties than the control. The industrial development of fortification of yogurt with small amounts of CW-SCG will require further studies to evaluate consumer acceptance and storage stability.

Key Words: rheological properties, texture, fortification

160 Valorization of Greek yogurt acid whey using filtration and acid-catalyzed lactose hydrolysis. M. Lindsay*[1], M. Molitor[3], K. Huang[1], C. Maravelias[1], J. Dumesic[1], S. Rankin[2], and G. Huber[1], [1]*Department of Chemical and Biological Engineering, University of Wisconsin-Madison, Madison, WI*, [2]*Department of Food Science, University of Wisconsin-Madison, Madison, WI*, [3]*Center for Dairy Research, University of Wisconsin-Madison, Madison, WI.*

This study uses a novel, catalytic approach to convert Greek yogurt acid whey (GAW) into 3 different products: 1) whey protein, 2) milk minerals, and 3) glucose-galactose syrup. Milk minerals, a high-value calcium supplement, is produced at higher yields in GAW compared to sweet whey due to the higher soluble calcium in GAW. Glucose-galactose syrup is a sweetener syrup which could replace other sweeteners such as high fructose corn syrup. The process to produce these products involves various filtration methods to separate the protein, salt, and other non-lactose components from the GAW, followed by acid-catalyzed lactose hydrolysis. The hydrolyzed product undergoes neutralization, filtration by anion exchange resin and activated carbon, and evaporation to produce a sweet brown syrup with similar composition and sweetness to high fructose corn syrup. A full kinetic model was developed on acid-catalyzed lactose hydrolysis to allow for design and optimization of industrial hydrolysis reactors. We have demonstrated this technology at the pilot scale (>100 gallons/hr) at the Center for Dairy Research at Babcock Hall in UW-Madison. An economic analysis was conducted to determine the potential economic viability of the technology. The results of the economic analysis indicate that Greek yogurt producers could have an additional revenue of over $11 million per year with a 26% rate of return by applying this technology.

Key Words: waste reduction, catalysis, filtration

161 Effect of nanopowdered eggshell on the characteristics of probiotic yogurt. D. G. Kamel*, *Dairy Science Department, Assiut University, Assiut, Egypt.*

Eggshell (ES) is a waste product of the food industry that could increase environmental pollution. ES is an alternative, cheap, and bioavailable source of dietary calcium (Ca) that can be utilized to fortify the Ca content of probiotic yogurt using nanotechnology. The calcium content of commercial yogurt is limited to 190.0 mg $100g^{-1}$. The objective of this study was to produce probiotic yogurt (5.0–7.0 log cfu g^{-1}) with high Ca content by fortification with nano-sized (0.02, 0.04, and 0.06 mg mL^{-1}) ES powder (ESP). Nano-sized ESP was prepared by milling pre-boiled dried ES using mortar grinder Fritsch Pulverisette 2. The size of the milled powder was measured using PW 1700 X-ray diffractometer to ensure that the diameter of the nano-sized is 25 ± 1.7 nm. Yogurt was manufactured by dividing the pasteurized milk into 4 aliquots portions to make the yogurt. The first portion (T1), second (T2), third (T3), and fourth (T4) portions were inoculated with 1% *Lactobacillus delbruckii* subsp. *bulgaricus* (Lb), 1% *Streptococcus thermophilus* (St), and 15% *Bifidobacterium bifidum* (Bb). T1 was considered as control while 0.02, 0.04, and 0.06 mg mL^{-1} of nano-sized ESP were added to T2, T3, and T4, respectively. All treatments were inoculated at 40°C until a pH of 4.6 was reached. Subsequently, the yogurt was cooled and stored at 4°C for 16 d. The acidity, Ca, sensory properties, Bb count, total bacterial count (TBC), yeast and mold counts were examined. This experiment was repeated 3 times using 3 different batches of raw milk. The addition of nano-sized ESP decreased acid development significantly ($P < 0.05$). The TBC significantly decreased ($P < 0.05$) to 6.1 log cfu g^{-1} as the concentration of nano-sized ESP increased to 0.06 mg mL^{-1}. The addition of 0.06 mg mL^{-1} nano-sized ESP produced a high Ca (705.4 mg $100g^{-1}$) yogurt with an acceptable composition and quality as compared with control (197.6 mg $100g^{-1}$). All sensory evaluation showed that the addition of nano-sized ESP improved taste, appearance, body and texture of probiotic yogurt. Moreover, the addition of nano-sized ESP increased the shelf-life of probiotic yogurt as compared with control. The impact of nano-sized ESP in probiotic yogurt during storage on the nutritional benefits will be evaluated in subsequent studies.

Key Words: nanopowdered eggshell, functional yogurt, shelf life

Forages and Pastures

162 Meta-analysis of the effects of preservatives on hay spoilage II: Microbial inoculants. M. Killerby*[1], R. White[2], D. C. Reyes[1], A. Y. Leon-Tinoco[1], S. Rivera[1], H. Paz[3], J. A. Jendza[4], and J. J. Romero[1], [1]*Animal and Veterinary Sciences, School of Food and Agriculture, University of Maine*, *Orono, ME*, [2]*Animal and Poultry Sciences, Virginia Polytechnic Institute and State University*, *Blacksburg, VA*, [3]*Department of Animal and Dairy Sciences, Mississippi State University*, *Starkville, MS*, [4]*BASF*, *Florham Park, NJ*.

Our objective was to conduct a meta-analysis to evaluate the effects of preservatives on hay spoilage during storage. A literature search was conducted using the ISI Web of Science database. Microbial inoculants (82 treatments from 21 articles) were analyzed as a separate subset from chemical preservatives due to incompatibilities in application rate units (log cfu/fresh g vs. %, respectively). The metafor package of R statistical software was used to fit a multilevel linear mixed-effects model with response variables calculated as standardized mean differences (SMD) between treated and untreated samples within an experiment, within a study. Responses included DM loss, sugars, visual relative moldiness (MP) and hay maximum temperature (MT). Experiment and study were random effects. Moderators included forage type (FT: grass, legume, or mix); moisture class [MC: < 20% (low moisture) or > 20% (high moisture)] and application rate (AR). The 2- and 3-way interactions among moderators were also tested. Final models were selected using a backward selection procedure where non-significant variables were removed iteratively. In low moisture hay, predicted DM losses increased by 2.61% units for each 2 log cfu/fresh hay g increase in AR but no changes were observed in high moisture hay ($P = 0.005$), which had an overall greater predicted DM losses relative to low moisture hay (7.25 vs. 2.57%; $P < 0.001$). Sugar concentration was higher in treated grasses compared with legumes (SMD = 7.78 vs −1.10; $P < 0.001$). Furthermore, predicted sugar concentration increased by 1.32% DM units in high moisture hay and decreased by 0.387 in low moisture hay for each 2 log cfu/g increase in AR ($P < 0.001$). Predicted MP increased by 17.1% units for each 2 log cfu/g increase in AR across both moisture classes ($P < 0.001$) and a higher MP was observed in high vs. low moisture hay (41.2 vs. 14.0%; $P < 0.001$). In high moisture hay, legumes had a higher predicted MT than grasses (47.0 vs. 39.3°C) but in low moisture hay an opposite trend was observed (26.7 vs. 33.8; $P = 0.001$). High moisture legume hay is more susceptible to spoilage than grasses and inoculants evaluated failed to prevent spoilage.

Key Words: hay, inoculants, meta-analysis

163 The use of a silage inoculant for sustainable milk production. I. Eisner*[1], K. Witt[1], L. Vandaele[2], J. De Boever[2], and G. Copani[1], [1]*Chr. Hansen Animal Health and Nutrition*, *Hørsholm, Denmark*, [2]*Flanders Research Institute for Agriculture, Fisheries and Food (ILVO)*, *Melle, Belgium*.

Optimal use of roughages on dairy farms can save concentrates, improve farm nutrient balance, increase dairy profitability and contribute to the global sustainable milk production. The objective of this study was to investigate the impact of a concentrate reduction in combination with inoculated roughages on feed intake and milk production. Grass silages from 1st and 2nd cut and whole plant maize silage were prepared in side by side bunkers. Control (CON) silages were ensiled without inoculant. Experimental silages were inoculated with SiloSolve FC (FC) containing 50:50 of *Lactobacillus buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037) at 1.5×10^5 cfu/g of fresh forage. A crossover feeding trial (2 periods x 4 weeks) with 32 mid-lactation Holstein dairy cows (balanced randomization procedure) investigated the impact on feed intake and milk production. The data were analyzed using a GLM (period * treatment + DIM + (1|cow)). The basal ration of the CON group consisted, on dry matter (DM) basis, of 40% CON maize silage, 40% CON grass silage, 9% sugar beet pulp silage and 11% of a barley/corn mixture. The cows were individually supplemented with concentrates in the feeding stations according to their stage of lactation and milk production. Cows in the FC group were fed the same basal ration but including FC silages and 0.5 kg/head (0.4 kg DM) less concentrate in the feeding stations. The FC cows completely compensated the lower concentrate intake by a 0.4 kg higher roughage DM intake and tended to produce 0.3 kg more milk (34.0 vs 33.7, $P = 0.099$) with a 0.1% lower fat content (4.21 vs 4.31, $P < 0.05$), so that the production of fat and protein corrected milk was unaffected. These results demonstrate that the inclusion of the inoculant treated maize and grass silages in the diet of dairy cows allowed for a reduction of daily concentrate intake by 0.4 kg DM without negatively affecting fat and protein corrected milk yield.

Key Words: silage inoculant, concentrate intake, milk production

164 Fermentation parameters and microbiome dynamic of whole-plant corn silage inoculated with *Lactobacillus buchneri* or in combination with *Lactobacillus hilgardii* and *Pediococcus pentosaceus*. P. Drouin[1], R. Scuderi*[1], and E. Apper[2], [1]*Lallemand Specialties*, *Milwaukee, WI*, [2]*Lallemand SAS*, *Blagnac, France*.

Aerobic deterioration is a key concern in silage of sugar-rich forage having low buffering capacity, as it can cause low quality or important losses during feed-out. Inoculation of silage with several microbial additives can result not only in limiting the aerobic deterioration but also in reducing losses. The objective of the trial was to evaluate inoculant usage on fermentation losses, fermentation parameters and aerobic stability (AS) of the silage. An ensiling trial (7-L mini-silos) was performed using a conventional hybrid corn silage. The treatments consisted of tap-water (Control), *L. buchneri* (LB)(Lalsil Fresh HC), and a combination of 2 heterofermentative strains, *L. buchneri, L. hilgardii*, and the homofermentative *P. pentosaceus* (Combo)(Lallemand Magniva Platinum 3). Openings were at 30, 60, and 180 d. Fermentation parameters, organic volatile compounds, AS, and nutritional parameters were evaluated. Bacterial composition was also determined using 16S rRNA amplicon sequencing. Design was a randomized complete block, blocked by repetition. One-way ANOVA was performed for each opening. At all 3 openings, lactic acid concentration was similar for Combo and Control silages and lower in LB silage. Lactate/acetate ratio was higher for Combo compared with Control silage. Results obtained with the Combo were indicative of homofermentation. AS was over 240 h by first opening for LB and Combo silages, lower in the control group at 30 and 60 d opening ($P < 0.001$). 1,2-Propanediol was highest in LB, and lowest in Control silage ($P < 0.001$). Propionic acid was below detection level. Compared with LB, the Combo significantly decreased losses of fresh matter for all 3 openings ($P < 0.001$ each openings). Dry matter recovery follows the same trend ($P = 0.009$). Mean NDF of fresh forage was of 38.8% DM ($P = 0.858$), but down to 32.5% 30.4% for Control and Combo ($P = 0.197$). The results will be compared with the bacterial microbiome, which was dominated by *Lactobacillaceae*. This trial confirmed the use of different bacterial strains improves AS by limiting fermentation losses.

Key Words: microbiome, silage, bacterial additives

165 Effect of microbial inoculation on fermentation profile and aerobic stability of rye silage harvested at different stages of maturity. I. Eisner*[1], K. Witt[1], V. Vrotniakiene[2], J. Jatkauskas[2], and G. Copani[1], [1]*Chr. Hansen Animal Health and Nutrition*, *Hørsholm, Denmark*, [2]*Institute of Animal Science of Lithuanian University of Health Sciences*, *Baisogala, Lithuania*.

The possibility to harvest a rye crop at a boot stage before corn planting may increase the total forage yield per hectare. If the crop is used for biogas production, harvest can take place at other stages of maturity.

The objective of this study was to evaluate the effects of a microbial inoculant on fermentation parameters and aerobic stability of rye silage. Rye was harvested at 4 stages of maturity. The crop was ensiled immediately at the flowering, milk, and soft-dough stage. At boot stage, rye was ensiled immediately (BW) or after 24 h of field wilting phase (BD). Five mini-silos (3 l volume) for each treatment were filled with crop (193 kg DM/m^3). Control forage (CON), with no inoculant, and experimental treatment (SSFC), inoculated with SiloSolve FC (150,000 total cfu/g of forage), containing 50:50 of *Lactobacillus buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037), were stored for 60 d at 20°C. After fermentation, each mini-silo followed an aerobic stability (AS) test by monitoring the temperature increase inside the mini-silos (duration: until they reached +3°C above T ambient, varying from 7 to 30 d depending on crop stage). Data were analyzed in a randomized block design using the GLM procedure (SAS, 9.4) with inoculum as a fixed effect for each maturity stage separately. The use of SSFC significantly enhanced the acetate levels at all maturity stages ($P < 0.05$). The concentration of butyric acid in SSFC silage was 2.0; 1.4; 0.4; 1.6; 0.7 vs. 9.4; 7.0; 5.6; 11.0; and 6.9 g/kg DM in CON silage for BW, BD, flowering, milk, and soft-dough stage respectively. The observed differences were significant at all stages of maturity ($P < 0.01$). AS was significantly improved in SSFC silage at all stages of maturity ($P < 0.05$). The numerically biggest difference in AS was achieved at BD phase (688 vs.120 h for SSFC and CON respectively; $P < 0.01$). These results demonstrate that SiloSolve FC was beneficial for both fermentation and aerobic stability, providing a flexible solution to direct ensiling of rye from boot till soft-dough maturity stage.

Key Words: aerobic stability, silage inoculant, rye

166 Artificial wilting in a forced-air oven has minimal effects on silage fermentation and quality in both inoculated and untreated vacuum-bag mini-silos. A. Wilder* and S. Bosworth, *University of Vermont*, *Burlington, VT*.

The dry matter content of forage material at ensiling is known to have substantial effects on silage fermentation and quality and is difficult to control when different forages are ensiled for comparison. Conventional wilting practices are unpredictable due to weather variability and a method for consistently wilting forages artificially could be beneficial for fermentation experiments utilizing multiple forage types and sources. We hypothesize that forage material may be wilted in a controlled manner in a forced-air oven at 55°C with little effect on silage fermentation and quality. To this end, 2 experiments were conducted comparing field wilting and oven wilting using alfalfa and red clover forage material. Silage inoculation was also evaluated to determine if the artificial wilting temperature increased the necessity of inoculation before ensiling. Replicated vacuum-bag mini-silos were used and samples were taken after 60 d to determine the fermentation profile and NIR forage quality of the ensiled forage material. Results were analyzed using a factorial ANOVA model. Artificial wilting only resulted in minor differences in silage fermentation and quality and, although inoculation consistently reduced silage pH, the lack of interactions with wilting method suggests that the inoculant functioned similarly in both cases.

Key Words: artificial wilting, silage fermentation, silage inoculant

Table 1 (Abstr. 166). Measures of silage fermentation and quality (g kg^{-1} DM unless otherwise noted)

	Field Wilted		Oven Wilted			Probability > F		
Measure	Inoc.	Ctrl.	Inoc.	Ctrl.	SE	Wilting	Inoc.	Wilting × Inoc.
1st-Cut Alfalfa								
DM content (g kg^{-1} silage)	462	444	445	434	6.6	0.0426	0.0255	NS
Silage pH	4.52	5.13	4.56	4.99	0.05	NS	<0.0001	NS
Lactic acid	69	71	74	75	1.9	NS	NS	NS
Acetic acid	11	15	15	26	2.2	0.0015	0.0021	NS
Ammonia	19	23	22	25	1.8	0.0218	0.0051	NS
Total VFA	80	86	89	100	2.9	0.0002	0.0015	NS
2nd-cut red clover								NS
DM content (g kg^{-1} silage)	463	459	436	435	9.7	NS	NS	NS
Silage pH	4.36	4.53	4.32	4.46	0.04	0.0475	0.0001	NS
Lactic acid	54	36	60	39	3.1	NS	0.0001	NS
Acetic acid	16	24	17	27	1.8	NS	0.001	NS
Ammonia	10	12	9	11	0.6	0.0162	0.0063	NS
Total VFA	70.0	59.8	77.2	65.3	4.09	NS	0.0146	NS

167 The effects of a silage inoculant on fermentation parameters in big bales and mini-silos. I. Eisner*[1], K. Witt[1], V. Vrotniakiene[2], J. Jatkauskas[2], and G. Copani[1], [1]*Chr. Hansen Animal Health and Nutrition*, *Hørsholm, Denmark*, [2]*Institute of Animal Science of Lithuanian University of Health Sciences*, *Baisogala, Lithuania*.

Mini-silos are widely used for testing the effectiveness of silage inoculants in the laboratory conditions with the assumption that similar effects are observed in the field. The objective of this study was to investigate the effect of an inoculant on fermentation characteristics in whole plant corn silage prepared in mini-silos and in round big bales. Crop was harvested at 35% dry matter (DM), inoculated with distilled water (C) or Biomax (BM) containing 50:50 of *Lactobacillus plantarum* (DSM26571) and *Enterococcus faecium* (DSM22502) (150,000 total cfu/g of fresh forage). Five round big bales (BB) (800 kg/bale) and 5 mini-silos (MS) (3 l of volume; 200 kg DM/m^3) for each treatment were prepared. All silages were stored for 120 d. Big bales were stored outside (−3°C till 14°C) and mini-silos at room temperature (20°C). The data were analyzed as a completely randomized design using PROC GLM of SAS 9.3 (fixed effects: inoculum, method and their main interaction). BM significantly increased lactic acid (64 vs. 47 g/kg DM; $P < 0.001$), decreased butyric acid (0.02 vs. 0.76 g/kg DM; $P < 0.001$) and pH (3.87 vs. 4.01; $P < 0.001$). Method had a significant effect on total acids, alcohols and DM loss ($P < 0.05$). Compared with C, BM increased total acids in BB (74 vs. 63 g/kg DM; $P < 0.002$) and in MS (85 vs. 69 g/kg DM; $P < 0.001$). Alcohols were lower in BM silage in BB (7.1 vs. 11.1 g/kg DM; $P < 0.001$) and in MS (8.0 vs. 12.4 g/kg DM; $P < 0.001$). BM reduced dry matter loss in BB (57 vs. 91 g DM; $P < 0.001$) and in MS (40 vs. 69 g/DM). An interaction between treatment and method was found for acetic acid ($P < 0.001$) and ammonia-N ($P = 0.023$). BM reduced acetic acid in BB (11 vs. 16 g/kg DM; $P < 0.001$), but not in MS. Ammonia-N was lower in BM silage in BB (3.8 vs. 5.7% total N; $P < 0.001$) and in MS (3.4 vs. 4.6% total N; $P < 0.001$). Although the values for some fermentation parameters varied with the ensiling method (BB/MS), inoculation had positive effects on fermentation and dry matter preservation in both methods. The mini-silo technique can be used to predict the effect of a silage inoculant under field conditions.

Key Words: big bales, mini-silo, silage inoculant

168 Effect of lignosulfonates on the dry matter loss, nutritional composition, and microbial counts of high-moisture alfalfa silage.

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Our objective was to evaluate the effect of 2 technical lignins, sodium and magnesium lignosulfonate (NaL and MgL, respectively) and an inoculant mixture (INO) on high moisture alfalfa silage spoilage. Treatments were untreated (0%), NaL and MgL applied independently at 0.5, 1, and 1.5 (% wt/wt, fresh basis) and INO (*Pediococcus pentosaceus* and *Lactobacillus plantarum*; 5 and 4 log cfu/fresh alfalfa g, respectively). An alfalfa field was divided into 5 plots (blocks), and unwilted alfalfa was chopped, treated (randomly within block), and ensiled for 229 d in mini-silos (0.29 L). Data were analyzed as a randomized complete block design (5 blocks) and differences were declared at $P \leq 0.05$. Linear (L) and quadratic (Q) polynomial contrasts were used to determine dose rate effects for NaL and MgL and orthogonal contrasts for INO effects. At d 0, increasing NaL dose from 0 to 1.5% increased DM (21.9 to 22.7 ± 0.12%; L) and decreased mold counts (4.48 to 3.62 ± 0.142 log cfu/fresh g; L). No treatment effects were observed on yeast (6.94) and lactic acid bacteria counts (7.04 log cfu/fresh g), sugars (6.15), CP (21.7), and NDF (43.2% of DM). At opening (d 229), increasing the NaL dose from 0 to 1.5% increased pH (4.33 to 4.56 ± 0.018; L) and DM (18.9 to 19.9 ± 0.19%; L), and decreased lactic acid (8.80 to 7.13 ± 0.24% of DM; L) and the lactic to acetic acid ratio (2.39 to 1.78 ± 0.07; L) but did not affect DM losses (~11.8 ± 0.67%). MgL increased pH (4.33 to 4.45 ± 0.018; L) and DM losses (11.28 to 14.05 ± 0.67%; L), and decreased lactic acid (8.80 to 6.74 ± 0.24%). Neither NaL nor MgL had an effect on sugars (~0.67 ± 0.07%), NH_3-N (~10.9 ± 0.54% of N), acetate (~3.82 ± 0.13% of DM), and ethanol (~1.02 ± 0.04% of DM). Compared with untreated ($P \leq 0.05$), INO increased DM losses (11.28 vs. 13.7 ± 0.67%), pH (4.33 vs. 4.46 ± 0.018), and decreased lactic acid (8.8 vs. 7.37 ± 0.24%) and sugars (0.8 vs. 0.45 ± 0.07% DM). Across treatments, yeasts and molds were below 2 log cfu/fresh g. In conclusion, additives tested did not improve the preservation of high moisture alfalfa silage, with INO and MgL actually increasing DM losses relative to untreated.

Key Words: alfalfa, silage, lignosulfonates

Physiology and Endocrinology

169 Physically effective neutral detergent fiber content modulates chewing activity, rumen fermentation, plasma metabolites, and performance. Y. Cao*[1,2], X. Chen[2], L. Wang[1,2], and J. Yao[1], [1]*Northwest A&F University*, *Yangling, Shaanxi, China*, [2]*Harvard Medical School*, *Boston, MA*.

Subacute ruminal acidosis (SARA) continues to be a common and costly metabolic disorder in high-producing cows worldwide. To evaluate if increasing physically effective neutral detergent fiber (peNDF) in diet can prevent SARA in cows fed high concentrate diets. Thirty second-parity Holstein cows were randomly allocated to 3 treatment groups: H-peNDF$_{8.0}$, M-peNDF$_{8.0}$, and L-peNDF$_{8.0}$, which were prepared by mixing the same total mixed ration for 10, 18, or 60 min, respectively. The peNDF$_{8.0}$ intake was positively correlated with the peNDF$_{8.0}$ contents in the diets. Total chewing and ruminating time was lower for the L-peNDF$_{8.0}$ diet than for the H-peNDF$_{8.0}$ and M-peNDF$_{8.0}$ diets ($P < 0.05$). Rumen pH was higher in the H-peNDF$_{8.0}$-fed cows than in the other 2 groups ($P < 0.05$). The H-peNDF$_{8.0}$ and M-peNDF$_{8.0}$ diets corresponded with higher acetate concentration, acetate:propionate ratio than the L-peNDF$_{8.0}$ diet ($P < 0.05$), while H-peNDF$_{8.0}$ and M-peNDF$_{8.0}$ resulted in lower propionate and valerate concentrations than L-peNDF$_{8.0}$ ($P < 0.05$). Lowering the peNDF$_{8.0}$ content decreased the activities of ruminal carboxymethyl cellulase, avicelase, and β-glucanase ($P < 0.05$). H-peNDF$_{8.0}$ resulted in lower total plasma antioxidant capacity, γ-glutamyl transpeptidase, albumin, and creatinine compared with M-peNDF$_{8.0}$ and L-peNDF$_{8.0}$ ($P < 0.05$). Somatic cell counts in milk were positively correlated with the dietary peNDF$_{8.0}$ content. The feed and milk energy efficiencies were unaffected by the treatments. In conclusion, increasing the content of peNDF$_{8.0}$ in diet could help alleviate SARA and improve animal health among early lactation cows fed a high concentrate diet by increasing peNDF$_{8.0}$ intake, chewing activity, and rumen pH.

Key Words: physically effective NDF (peNDF), subacute ruminal acidosis (SARA), chewing activity

170 Hepatic lipid-associated protein abundances vary by day relative to calving and are associated with hepatic triglyceride content in transition dairy cows. H. T. Holdorf*, R. S. Pralle, S. J. Erb, and H. M. White, *University of Wisconsin-Madison*, *Madison, WI*.

The objectives of this study were to determine protein abundance of hepatic lipid-associated proteins (HLAP) in liver homogenates and interrogate a potential relationship with peripartum liver triglyceride (LVTG) content. Multiparous Holstein cows (n = 25) were blocked by expected calving date and randomly assigned to control or a fatty liver induction (FLI) treatment (TRT). Liver samples were collected via biopsy at −28, −14, +1, +14, +28, +42, and +56 d relative to calving (DRTC). Content of LVTG was determined and expressed on a % dry matter basis. Western blotting was used to analyze the abundance of HLAP: abhydrolase domain containing 5 (ABHD5), hormone sensitive lipase (HSL), phosphorylated HSL (PHSL), adipose triglyceride lipase (ATGL), perilipin 1 (PLIN) and phosphorylated PLIN (PPLIN). Data was analyzed using PROC MIXED in SAS 9.4. Evidence was considered significant if $P \leq 0.05$, and a tendency if $0.05 < P \leq 0.1$. When $P \leq 0.1$ for the main effects, means were separated by Bonferroni adjustment and presented as least squares means ± SEM with comparison P-values. No evidence was observed for effects of TRT or TRT × DRTC ($P > 0.1$) on LVTG content or HLAP abundance. Mean LVTG content was greater ($P \leq 0.05$) postpartum compared with prepartum and peaked at +14 DRTC ($P \leq 0.0004$). Greater ATGL abundance was observed ($P \leq 0.04$) at +14 and +28 DRTC (μ = 1.89 ± 0.02 arbitrary units [AU]) compared with +1 (1.83 ± 0.03 AU) DRTC. Abundance of PLIN tended to be greater ($P = 0.08$) at +14 (0.79 ± 0.02 AU) compared with +1 DRTC (0.72 ± 0.03 AU). Conversely, PPLIN was decreased ($P \leq 0.003$) at +1, +14, +28, and +42 (μ = 1.67 ± 0.02 AU) compared with −14 DRTC (1.74 ± 0.01 AU). As linear regressors, PPLIN predicted ($P = 0.05$; $\beta = -0.44$) and ATGL tended to predict ($P = 0.09$; $\beta = -0.33$) LVTG content across all DRTC. Variation in HLAP abundance by DRTC and their associations with LVTG suggest a potential role in the accumulation and subsequent remobilization of LVTG. The impact of coordinated regulation of HLAP on the etiology of fatty liver onset and recovery peripartum warrants further exploration.

Key Words: lipase, fatty liver, peripartum

171 Effects of hindgut acidosis in lactating dairy cows already experiencing systemic inflammation. M. A. Abeyta*, E. A. Horst, E. J. Mayorga, B. M. Goetz, S. J. Rodriguez-Jimenez, M. F. Caratzu, and L. H. Baumgard, *Department of Animal Science, Iowa State University*, *Ames, IA*.

Following parturition cows experience an increased starch load to the large intestine, and we hypothesize that the negative consequences of hindgut acidosis are exacerbated by prior periparturient immunoactivation. Therefore, objectives were to evaluate the effects of hindgut acidosis on metabolism and inflammation in cows previously infused i.v. with lipopolysaccharide (LPS). Twelve rumen cannulated cows were enrolled in a study with 3 experimental periods (P). Baseline data were collected during P1 (5d). Beginning on d1 of P2 (2d), all cows received an i.v. LPS bolus (0.2 μg LPS/kg BW). During P3 (4d), cows were randomly assigned to 1 of 2 abomasal infusion treatments: 1) control (LPSCON; 1.5 L H_2O/infusion; n = 6) or 2) starch infused (LPSST; 1 kg corn starch + 1.5 L H_2O/infusion; n = 6) 4 times daily. Additionally, both treatments received an LPS bolus on d1 and 3 of P3 (0.8 and 1.6 μg LPS/kg BW, respectively). Effects of treatment, time, and treatment × time were assessed using PROC MIXED. During P3, starch infusion markedly decreased fecal pH relative to controls (0.82 pH units; $P < 0.01$). Relative to P1, administering LPS decreased production metrics during both periods, and the most pronounced effects were observed on d1 of P2 and d1 and 3 of P3 for milk yield (54, 45, and 37%) and DMI (49, 43, and 40%) respectively (all $P < 0.01$); However, starch infusion did not exacerbate effects of LPS on either parameter. Regardless of starch infusion, administering LPS markedly altered milk components and somatic cell counts. During P3, hindgut acidosis had no effect on circulating glucose, insulin, NEFA, or BHBA ($P > 0.35$), but decreased BUN (17%; $P = 0.07$) relative to LPSCON cows. Relative to P1, LPS administration markedly increased SAA and LBP during P2 (3.3-fold and 51%) and P3 (2.7-fold and 48%; all $P < 0.01$), however there were no additional effects of starch administration during P3 on acute phase proteins. By design, LPS administration initiated marked changes in metabolism and inflammation, however, hindgut acidosis did not exacerbate the inflammatory response.

Key Words: inflammation, starch infusion, LPS

172 Effects of hindgut acidosis in feed-restricted dairy cows. M. A. Abeyta*, E. A. Horst, E. J. Mayorga, B. M. Goetz, S. J. Rodriguez-Jimenez, M. F. Caratzu, and L. H. Baumgard, *Department of Animal Science, Iowa State University*, *Ames, IA*.

Cows experience an increased starch load to the large intestine following parturition, and we hypothesize that the negative consequences of hindgut acidosis (HGA) may be exacerbated by prior periparturient stressors (i.e., reduced feed intake, inflammation). Therefore, objectives were to evaluate the effects of HGA on metabolism and inflammatory biomarkers in feed restricted (FR) cows. Twelve rumen cannulated cows were enrolled in a study with 3 experimental periods (P). During P1 (5d), baseline data were collected. During P2 (2d), all cows were FR to 40% of their ad libitum P1 feed intake. During P3 (4d) cows remained FR and were randomly assigned to 1 of 2 abomasal infusion treatments: 1) control (FRCON; 1.5 L H_2O/infusion; n = 6) or 2) starch (FRST; 1 kg corn starch +

1.5 L H_2O/infusion; n = 6). Respective treatments were infused 4 × daily. Effects of treatment, time, and treatment × time were assessed using PROC MIXED. Starch infusions markedly decreased fecal pH relative to FRCON during P3 (0.96 pH units; $P < 0.01$). By design, DMI decreased 60% relative to baseline for both treatments during P2 and P3 ($P < 0.01$). Milk yield was markedly decreased by FR during P2 and 3 relative to P1 (39%; $P < 0.01$), but was unaffected by HGA ($P > 0.91$). Feed restriction altered milk components and variables differently over time with no effect of HGA except on MUN, which decreased (28%; $P = 0.01$) relative to FRCON during P3. Feed restriction increased NEFA and decreased circulating glucose and insulin for both periods and the most pronounced differences were observed during P2 (3.6-fold, 13 and 74%, respectively; $P < 0.01$). Starch infusion did not influence circulating NEFA, glucose, and insulin patterns during FR. Relative to FRCON, BHBA concentrations increased (55%; $P = 0.04$) in FRST cows during P3. Over time, FR decreased BUN for both treatments but starch infusions further exacerbated this response during P3 as BUN levels decreased (31%; $P = 0.03$) relative to FRCON. No effects of FR or HGA were observed for SAA and LBP. By design, FR caused marked alterations in metabolism, however, no effects of FR or FR in combination with HGA were observed on inflammation.

Key Words: inflammation, starch infusion

173 Prepartum light shifting circadian rhythm disruption did not affect amount of muscle and adipose mobilized in transition dairy cows. C. J. McCabe*, A. Suarez-Trujillo, T. M. Casey, and J. P. Boerman, *Purdue University, Department of Animal Sciences*, *West Lafayette, IN*.

Circadian clocks function to maintain homeostasis by coordinating internal physiology to the external environment through the generation of 24 h rhythms. Circadian clocks are integrated with the metabolic system and disruption of clocks by altering timing of external cues affects metabolism, with long-term disruption associated with development of diseases in humans and rodents. During the periparturient period, dairy cattle are often in a negative energy balance and accommodate for nutrient gaps by mobilizing stores from adipose and muscle. The objective of this study was to determine the effect of exposure to chronic light-dark cycle phase shifts during the nonlactating prepartum period on tissue mobilization postpartum. Multiparous Holstein cows (n = 16) were exposed to 16 h of light and 8 h of dark (CON; n = 8) or phase shifting (PS; n = 8) of the start of the light cycle forward 6 h every 3 d beginning 35 d before expected calving (BEC). Following calving both treatments were exposed to control lighting through 60 DIM. Longissimus dorsi and backfat ultrasounds measured muscle and backfat depth at 35, 21, and 7 d BEC and 0, 10, 21, 30, and 60 DIM. Cows lost muscle mass from 35 d BEC to 60 DIM (4.02 vs. 3.01 cm; $P < 0.01$), with no differences between treatments ($P > 0.05$). Muscle depth at 35 d BEC positively correlated to the amount of muscle mobilized over the study ($P = 0.002$; $R^2 = 0.50$). Backfat depth was less between 35 d BEC and 30 and 60 DIM ($P < 0.05$). Creatinine, 3-methylhistidine, and NEFA, measured in plasma samples taken on ultrasound dates and 28, 14 d BEC and 7, 14 DIM as indicators of total muscle mass, and muscle and adipose mobilized, respectively, were not different between treatment ($P > 0.05$), but NEFA increased and creatinine decreased for all cows between pre and postpartum periods ($P < 0.01$). In this study, circadian clock disruptions in the prepartum did not affect the quantity of tissue mobilized nor blood metabolites during the dairy cow periparturient period.

Key Words: circadian rhythm, ultrasound, tissue mobilization

174 Effects of antioxidant supplementation on metabolism and inflammatory biomarkers in heat-stressed dairy cows. M. A. Abeyta*[1], M. Al-Qaisi[1], E. A. Horst[1], E. J. Mayorga[1], S. J. Rodriguez-Jimenez[1], B. M. Goetz[1], S. Carta[1], H. Tucker[2], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University*, *Ames, IA*, [2]*Novus International*, *St. Charles, MO*.

Study objectives were to evaluate if antioxidant supplementation (AP; AGRADO Plus 2.0; Novus International, St. Charles, Missouri) affects metabolism and inflammatory biomarkers in hyperthermic lactating dairy cows. Thirty-two cows were randomly assigned to 1 of 4 dietary-environmental treatments: 1) thermoneutral (TN) conditions and control (TNCON; n = 8), 2) TN conditions and AP (TNAP; n = 8), 3) heat stress (HS) and control (HSCON; n = 8), or 4) HS and AP (HSAP; n = 8). Before study initiation, cows were fed their respective diets for 30d and dietary treatments were top-dressed once daily. The trial consisted of 2 experimental periods (P); during P1 (4d) baseline data were collected. During P2 (7d) HS was artificially induced using an electric heat blanket (Thermotex Therapy Systems Ltd., Calgary, Canada). Effects of treatment, day, and treatment × day interaction were assessed using PROC MIXED. HS increased ($P < 0.01$) rectal, vaginal, and skin temperatures relative to TN controls and the largest differences were detected on d3–7 (1.2, 1.1, and 1.9°C, respectfully). On d2 of HS, AP supplementation decreased respiration rate (15%; $P = 0.01$) relative to HSCON. As expected, HS decreased ($P < 0.01$) milk yield and DMI during P2 and this was most pronounced on d4–7 (28, and 33%, relative to TN). Feed efficiency was increased by AP supplementation on d4–7 of HS relative to HSCON (15%; $P = 0.06$). DMI from HSAP cows tended to be decreased relative to HSCON cows during P2 (10%; $P = 0.06$). Circulating insulin and NEFA did not differ across environmental treatments, however, AP supplementation decreased insulin (37%; $P = 0.01$) and increased NEFA (68%; $P = 0.03$) during HS relative to HSCON cows. HS decreased circulating glucagon (26%; $P < 0.01$) relative to TN cows. Throughout P2, BUN from HS cows increased (21%; $P < 0.01$) relative to TN, and on d3 AP supplementation decreased BUN relative to HS controls (15%; $P < 0.01$). HS increased SAA and LBP relative to TN cows (78 and 59%, $P = 0.08$ and < 0.01) but neither variable was influenced by AP. Overall, AP supplementation appeared to alter metabolism but not inflammation during heat stress.

Key Words: heat stress, antioxidant, inflammation

Ruminant Nutrition: Carbohydrates and Lipids

175 Milk production responses of dairy cows to fatty acid supplements with different ratios of palmitic and oleic acid in low- and high-fat basal diets. A. M. Burch*[1], J. de Souza[2], and A. L. Lock[1], [1]*Michigan State University*, *East Lansing, MI*, [2]*Perdue AgriBusiness*, *Salisbury, MD*.

We evaluated the effects of fatty acid (FA) supplements with different ratios of palmitic (C16:0) and oleic (C18:1) acids in low and high fat basal diets on production responses of dairy cows. Thirty-six multiparous Holstein cows (50.2 ± 5.8 kg milk/d; 160 ± 36 d DIM) were used in a split-plot Latin square design. Cows were blocked by milk yield and allocated to a main plot receiving either a low fat (LF) basal diet ($n = 18$) containing cottonseed meal and cottonseed hulls or a high fat (HF) basal diet ($n = 18$) containing whole cottonseed. Diets were balanced for similar NDF (30.0% DM), starch (28.5% DM), and CP (17.5% DM). Within each plot a 3x3 Latin square arrangement of treatments was used with 3 21 d periods. Treatments were: 1) control (CON; no FA supplementation), 2) FA supplement containing 80% C16:0 + 10% C18:1 (PA), and 3) FA supplement containing 60% C16:0 + 30% C18:1 (PAOA). FA supplements were fed at 1.5% DM and replaced soyhulls in CON. The statistical model included the random effect of cow within basal diet, and the fixed effect of treatment, basal diet, period, and their interactions. Treatment by basal diet interactions were observed with FA treatments increasing lactose yield ($P = 0.01$) and tending to increase milk yield ($P = 0.14$) in LF but not in HF. Basal diet had no effect on DMI ($P = 0.66$) or milk yield ($P = 0.62$). Compared with LF, HF increased 3.5% FCM (46.7 vs. 50.2 kg/d; $P = 0.04$) and milk fat yield (1.65 vs. 1.83 kg/d; $P = 0.02$) and tended to increase ECM (47.1 vs. 50.0 kg/d; $P = 0.07$). Results for FA treatments are presented in the following order: CON, PA, and PAOA. PAOA decreased DMI (33.0, 33.1, 32.0 kg/d; $P < 0.01$). FA treatments increased 3.5% FCM (47.4, 48.9, 49.0 kg/d; $P < 0.01$), ECM (47.8, 49.0, 48.9 kg/d; $P < 0.01$), and milk fat yield (1.69, 1.76, 1.76 kg/d; $P < 0.01$) compared with CON but there was no difference between FA treatments. In conclusion, a high fat basal diet had positive production responses while the addition of fat supplements increased milk fat yield, 3.5% FCM, and ECM regardless of basal diet.

Key Words: fat supplementation, basal diet, milk fat

176 Evaluation of sweet potato flour addition partially replacing corn on milk yield and metabolism of dairy cows. M. A. Ollé*[1], C. F. Demarco[1], L. Oliveira[2], C. A. Oliveira[1], J. B. Cardoso[1], A. E. Anjos[1], A. D. C. Matos[1], A. A. Barbosa[1], J. O. Feijó[1], V. R. Rabassa[1], E. Schmitt[1], M. N. Corrêa[1], F. A. B. Del Pino[1], and C. C. Brauner[1], [1]*Núcleo de Pesquisa, Ensino e Extensão em Pecuária (NUPEEC), Universidade Federal de Pelotas (UFPel)*, *Pelotas, RS, Brazil*, [2]*IFSul Campus Visconde da Graça (CAVG)*, *Pelotas, RS, Brazil*.

Sweet potato (*Ipomoea batatas*) is a high source of starch feed (75.7%). This crops usually generate up to 30% of discards that are not marketing, becoming a potential ingredient as a coproduct to be added in a ruminant ration. Therefore, the objective of this study was to evaluate sweet potato flour addition on concentrate, partially replacing corn, on milk yield and metabolism of dairy cows. Twenty lactating multiparous Holstein cows 45 ± 15 DIM were randomly enrolled into 2 groups: cows receiving a standard diet with corn as an energy source ($n = 20$) and cows receiving a concentrate with 40% of sweet potato flour replacing corn ($n = 20$). Milk production was obtained daily by the sum of the morning and afternoon milkings throughout the experiment. Serum and plasma samples were collected every 3 d from all animals after morning milking through a coccygeal vein puncture, and analyzed for glucose, nonesterified fatty acid (NEFA), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), total protein (TP), albumin and urea concentrations. The experiment lasted 70 d, divided into 2 periods of 35 d each, 14 d for adaptation to diet and 21 d for collection, adopting the crossover design (2 treatment groups and 2 periods) and data were analyzed using NCSS software. There was no effect ($P > 0.05$) of carry-over on the treatments in any variables. The average daily milk production was similar ($P = 0.62$) between groups, 38.95 and 38.67 kg/day, respectively for control and sweet potato groups. Metabolic evaluations were all within the physiologic ranges and they were similar ($P > 0.05$) between groups glucose ($P = 0.11$); NEFA ($P = 0.58$); GGT ($P = 0.19$); AST ($P = 0.48$); TP ($P = 0.21$); albumin ($P = 0.14$). The concentration of urea was greater ($P = 0.001$) in the sweet potato flour group, 38.63 mg/dL as compared with 34.96 mg/dL to the control. In conclusion, corn substitution by sweet potato flour at 40% in lactating cow diets, did not affect milk yield and metabolism. Therefore, sweet potato flour can be a potential ingredient to be used for lactating diets of dairy cows.

Key Words: milk production, ruminant nutrition, starch

177 Effect of high-amylase corn silage on lactational performance and enteric methane emission in dairy cows. S. F. C. Welchez*, H. Stefenoni, A. Melgar, C. F. A. Lage, S. E. Räisänen, D. Wasson, M. E. Fetter, and A. N. Hristov, *The Pennsylvania State University*, *University Park, PA*.

This study investigated the effect of high-amylase corn silage on lactation performance, enteric methane (CH_4) emission, and rumen fermentation of lactating dairy cows. Following a 2-wk covariate period, 48 cows averaging (±SD): 121 ± 30 d in milk (DIM), 43 ± 9 kg/d milk yield (MY), and 620 ± 61 kg BW were blocked based on DIM, MY, and enteric CH_4 emission and randomly assigned to 1 of 2 treatments in an 8-wk randomized complete block design experiment: (1) control corn silage from an isoline parental corn without α-amylase trait (CON) and (2) corn silage containing a bacterial transgene expressing high levels of α-amylase in the endosperm of the grain (EFC; EnogenFeed, Syngenta Seeds LLC, Downers Grove, IL). Silages were included at 40% of the total mixed ration dry matter (DM) EFC and CON silage were 43.3 and 41.8% DM and had (%, DM): neutral-detergent fiber (NDF), 36.7 vs. 37.5 and starch, 36.1 vs. 33.1, respectively. The overall diets contained (%, DM basis): crude protein, 16.5 vs. 16.7; NDF, 33.6 vs. 33.9; and starch, 25.2 vs. 24.0 for EFC and CON, respectively. Enteric CH_4 emission was measured using the GreenFeed system (C-Lock Inc., Rapid City, SD). Rumen samples were collected using the ororuminal sampling technique on experimental wk 6. Data were analyzed using PROC MIXED of SAS with block and block × treatment as random effects. DMI (25.3 kg/d; SEM = 0.34, $P = 0.55$) was similar between treatments. Compared with CON, MY (38.8 vs. 40.8 kg/d; SEM = 0.50), feed efficiency (1.53 vs. 1.63 kg/kg; SEM = 0.027), ECM feed efficiency (1.51 vs 1.57 kg/kg; SEM = 0.022), milk true protein (1.20 vs 1.25 kg/d; SEM = 0.033), and lactose yields (1.89 vs 2.00 kg/d; SEM = 0.016) were increased ($P \leq 0.05$) by EFC. Daily CH_4 emission (416 g/d; SEM = 8.2) was not affected ($P = 0.32$) by treatment, but CH_4 emission intensity was decreased ($P = 0.007$; 11.1 vs 10.3 g/kg milk; SEM = 0.22;) by EFC. Molar proportion of butyrate was higher ($P = 0.04$) in CON vs. EFC (14.6 vs. 11.3 mol %; SEM = 1.06). EFC inclusion at 40% of dietary DM increased milk yield and feed efficiency and decreased CH_4 emission intensity in dairy cows.

Key Words: dairy cow, corn silage, enteric methane

178 Milk production and feed intake responses to increasing levels of palmitic acid supplementation in early lactation Holstein cows. P. D. French*[1] and M. J. Martin[2], [1]*PHD R&D LLC*, *Fort Atkinson, WI*, [2]*University of Wisconsin*, *Madison, WI*.

Several published studies show that mid-lactation Holstein cows of varying production levels respond to increasing levels of palmitic acid (PA) supplementation. We evaluated the dose-dependent response of a PA supplement in early lactation Holstein cows fed a basal diet containing 2.6% total fatty acids (FA) and 0.5% PA. Twelve multiparous, Holstein cows (53 ± 15 DIM) with a preliminary milk yield of 62 ± 8 kg/d were fed a

PA supplement (88.0% C16:0, 3.6% C18:0, 5.1% C18:1) at 0, 0.5, 1.0, or 1.5% of ration DM in a replicated 4 × 4 Latin square design. Periods were 14 d with the final 5 d used for data collection except for milk composition and yield which was determined on the last 2 d of each period. Cows were milked 3x/d and fed a TMR once daily that contained 54% forage (35% corn silage and 19% alfalfa silage), 15.8% CP, 27.7% NDF, and 28.4% starch. Data were analyzed using the mixed model procedure of SAS and linear (L), quadratic (Q), and cubic (C) contrasts were used to evaluate PA dose-response. Results are presented in the following order: 0, 0.5, 1.0, or 1.5% PA. PA intake was 151, 297, 436, and 573 g/d. PA dose increased milk yield (60.1, 59.9, 61.4, and 61.6 kg/d; L, $P < 0.01$) and ECM (59.8, 58.9, 61.8, and 60.9 kg/d; L, C, $P < 0.01$). PA dose affected DMI (30.1, 31.6, 31.6, and 31.5 kg/d; Q, $P < 0.05$) and ECM feed efficiency (1.94, 1.88, 1.97, and 1.95; C, $P < 0.01$). Milk fat concentration (3.52, 3.44, 3.59, 3.53%; C, $P < 0.05$), milk FA acid concentration (3.33, 3.26, 3.40, and 3.34; C, $P < 0.05$) and milk fat yield (2.16, 2.10, 2.26, 2.20 kg/d; C, $P < 0.01$) were affected by PA dose. PA did not alter milk protein concentration (2.90%) but did affect yield (1.78, 1.77, 1.84, and 1.79 kg/d; C, $P < 0.01$; L, $P < 0.08$). Preformed FA (>C18) increased with PA dose (L, $P < 0.05$), whereas mixed FA (C16) responded cubically ($P < 0.05$), and de novo (<C16) were not affected by PA dose. In conclusion, high-producing Holstein cows responded most efficiently to 1.0% PA supplementation.

Key Words: fat supplementation, feed efficiency, milk fat

179 Effect of a live yeast supplement and altered ruminal fermentability of dietary starch on the yields of milk and milk components of mid- to late-lactation dairy cows. U. Abou-Rjeileh*, A. N. Negreiro, and A. L. Lock, *Michigan State University*, *East Lansing, MI.*

We evaluated the effect of a live yeast supplement and changes in ruminal fermentability of dietary starch on feed intake and the yields of milk and milk components of mid- to late-lactation dairy cows (158 ± 76 DIM). Thirty-two multiparous Holstein cows (45.5 ± 5.1 kg/d) were used in a crossover design with two 28-d periods. A fermentable starch challenge (FSC) on the last 7 d of each period was utilized as a split-plot within period. Cows were blocked by milk yield and randomly assigned to 1 of 2 treatments. Treatments were: 1) control diet with no live yeast supplementation (CON); and 2) diet supplemented with a live yeast supplement (*Saccharomyces cerevisiae* from Phileo by Lesaffre) at 0.03% diet DM (YEAST). Diets were formulated to contain (% DM) 19.8% NDF, 28.8% starch, 16.9% CP, and 4.5% fat. Diets containing a 2:1 ratio (DM basis) of dry ground corn (DGC) to high-moisture corn (HMC) were fed from d 1–21 of each period. During the last 7 d of each period the ratio of DGC to HMC was switched to a 1:2 ratio to increase ruminal starch fermentability. Response variables were averaged for d 17–21 (before the FSC period) and d 27 and 28 (during the FSC period). The statistical model included the random effect of cow and fixed effects of treatment, period, challenge, and their interactions. There was no effect of treatments on DMI or the yields of milk and milk components (all $P > 0.13$). While there was no main effect of the FSC on DMI or the yields of milk and milk components (all $P > 0.30$), effects on milk fat content and yield varied for CON and YEAST. During the FSC, milk fat yield decreased from 1.69 to 1.62 kg/d for CON but was not different for YEAST (1.68 to 1.66 kg/d; interaction $P = 0.06$). Milk fat content decreased from 3.55 to 3.39% for CON but was not different for YEAST (3.51 to 3.47%; interaction $P = 0.05$). Overall, the live yeast supplement did not impact DMI or the yields of milk and milk components. Results demonstrate that a live yeast supplement may help prevent milk fat depression during transition to a diet with increased ruminal starch fermentability.

Key Words: live yeast, starch, milk fat

180 Effects of timing of a calcium salt supplement containing palmitic and oleic acids on production responses of early lactation dairy cows. A. Pineda[1], J. de Souza*[2], J. Newbold[3], R. M. Kirkland[4], and A. L. Lock[1], [1]*Michigan State University*, *East Lansing, MI*, [2]*Perdue AgriBusiness*, *Salisbury, MD*, [3]*Scotland's Rural College*, *Dumfries, UK*, [4]*Volac Wilmar Feed Ingredients*, *Royston, UK.*

Fifty-six multiparous cows were used in a randomized complete block design experiment and assigned to either a control diet containing no supplemental fat (CON) or a diet supplemented with calcium salts (CS) of palmitic (C16:0) and oleic (C18:1) acids (FAT) either from calving to 24 DIM (fresh period, FR) or from 25 to 67 DIM (peak period, PK). Fresh diets contained (% DM) 17% CP, 23% forage NDF, and 25% starch. Peak diets contained (% DM) 17% CP, 19% forage NDF, and 28% starch. The CS contained 60% C16:0 and 30% C18:1 (total fatty acid basis) and was added at 1.90% of diet DM replacing soyhulls in CON diets. The statistical model included the random effect of block and cow within block and treatment, and the fixed effect of treatment, time, and their interactions. Results are presented in the sequence CON vs. FAT. During FR there was no effect of treatment on DMI (21.2 vs. 21.5 kg/d, $P = 0.25$), milk yield (40.6 vs. 39.1 kg/d, $P = 0.30$), or BW change (−2.59 vs. −2.20 kg/d, $P = 0.35$). Compared with CON, FAT increased milk fat content (4.62 vs. 4.95%, $P = 0.02$) and yield (1.80 vs. 1.96 kg/d, $P = 0.02$) and ECM (45.6 vs. 48.4 kg/d, $P = 0.05$). During PK there was no effect of treatment on DMI (28.1 vs. 28.3 kg/d, $P = 0.73$), or BW change (0.39 vs. 0.30 kg/d, $P = 0.38$). Compared with CON, FAT increased milk yield (51.5 vs. 54.0 kg/d, $P = 0.05$), milk fat content (3.52% vs. 3.63, $P = 0.05$) and yield (1.82 vs. 2.02 kg/d, $P < 0.01$) and ECM (51.5 vs. 55.2 kg/d, $P < 0.01$). A treatment by time interaction was observed for DMI during PK ($P = 0.05$) because intake was higher for FAT compared with CON after wk 5 of calving. We observed that cows that received FAT compared with CON during FR increased milk yield by 2.7 kg/d ($P = 0.04$) and ECM by 2.9 kg/d ($P = 0.03$) during PK indicating a positive carryover effect of CS supplementation early postpartum. Our results demonstrate that feeding a CS supplement containing 60% C16:0 and 30% C18:1 during early lactation increases milk fat yield and ECM without changes in DMI or BW during the fresh and peak periods.

Key Words: palmitic acid, oleic acid, postpartum

Ruminant Nutrition: Gut Physiology, Fermentation, and Digestion

181 Impact of combinations of dietary palmitic, stearic, and oleic acids on rumen fermentation and fiber digestibility.

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Previous research demonstrated that palmitic and/or oleic acid enhanced fiber digestibility. Since palmitic, stearic, and oleic acids are the main fatty acids (FA) present in ruminal bacteria cells we hypothesized that readily available 16- and 18-carbon FA from the diet are incorporated into rumen bacterial membranes supporting growth. Thus, our objective was to evaluate the effect of combinations of palmitic, stearic, and oleic acid on fiber digestibility and rumen fermentation. Continuous culture fermenters ($n = 8$) were used in a replicated 4 × 4 Latin square design. Treatments were: 1) Control diet with no supplemental FA (Total FA: 2.2%; Control); 2) Control diet plus 0.9 g of palmitic acid (Palmitic); 3) Control diet plus 0.29 g of palmitic, 0.56 g of stearic, and 0.05 g of oleic acid (Pal/Ste/Ole); and 4) Control diet plus 0.83 g of stearic, and 0.07 g of oleic acid (Ste/Ole). All FA treatments were supplemented at 1.5% DM diet. Treatment 2 was the positive control; treatment 3 mimicked the bacterial phospholipid 16- and 18-carbon FA profile, while treatment 4 mimicked the bacterial phospholipid 18-carbon FA profile. The control diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate mixture that provided 8.7 g CP, 21 g NDF, 11 g starch, and 1.5 g FA fed twice daily. Data were analyzed using a mixed model including the fixed effect of treatments, and the random effects of period and fermenter. Data are reported as least squares means with differences declared at $P \leq 0.05$. Three pre-planned contrasts were tested: 1) Control vs. Pal/Ste/Ole; 2) Pal/Ste/Ole vs. Ste/Ole; and 3) Pal/Ste/Ole vs. Palmitic. Compared with control, Pal/Ste/Ole increased NDF digestibility (43.8 vs. 39.5%, $P = 0.05$). Furthermore, Pal/Ste/Ole increased NDF digestibility compared with Ste/Ole (43.8 vs. 36.2%, $P < 0.01$). Compared with control, Pal/Ste/Ole increased total VFA production (198 vs. 169 mmol/d, $P = 0.04$). Ammonia concentration was not affected by treatments. Our preliminary results indicate that supplying dietary fatty acids that mimics the bacterial phospholipid 16- and 18-carbon FA profile favors ruminal fiber digestibility and VFA production.

Key Words: fatty acids, rumen, fiber

182 Effects of duodenal casein and glutamic acid infusion on small intestinal starch digestion and energy and nitrogen balance in cattle.

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Small intestinal starch digestion (SISD) is limited in cattle, but it can be improved by postruminal flow of casein or glutamic acid. The objective of this experiment was to evaluate effects of postruminal flow of casein or Glu on SISD and to quantify improvements in energy and nutrient balance. Twenty-two steers (BW = 179 ± 4 kg) were duodenally infused with raw cornstarch (1.46 ± 0.04 kg/d) and either 413 ± 7.0 g casein (CAS)/d, 121 ± 3.6 g Glu /d or water (CON). Measures of SISD and nutrient excretion were collected across 4 d after 42 d of infusion and measures of respiration via indirect calorimetry were collected across 2 d after –48 d of infusion. Ileal starch flow was least ($P = 0.05$) among calves provided CAS, but ileal starch flow was not different ($P = 0.60$) between Glu or CON. Correspondingly, SISD tended ($P = 0.11$) to be greatest among calves provided CAS, compared with Glu or CON. Casein increased ($P < 0.01$) ileal flow of ethanol-soluble starch compared with Glu and CON. Large intestinal starch digestion was similar ($P = 0.39$) across treatments. By design, N intake ($P < 0.01$) was greatest among cattle provided CAS, intermediate among calves provided Glu and least for CON. Nitrogen retention was greater ($P < 0.01$) in response to CAS compared with CON and Glu. Intake of GE from feed was similar ($P = 0.75$) across treatments, and GE from infusate was greatest for CAS, intermediate for Glu and least for CON. Variation in GE intake from feed resulted in no difference in overall GE intake ($P = 0.49$) across treatments. Similar to measures of SISD and N retention, CAS increased DE ($P = 0.04$) and ME ($P = 0.02$), compared with Glu and CON, which did not differ ($P > 0.20$). Postruminal infusions did not influence ruminal methane production ($P = 0.90$), but heat production tended to be greater ($P = 0.06$) among steers infused with CAS. Overall, amounts of energy retained by CAS tended ($P = 0.12$) to be nearly 34% greater than CON, but Glu had no impact on energy balance ($P = 0.43$). Improvement in SISD in response to CAS increased energy and N retained; however, Glu did not influence SISD and energy or N balance in cattle.

Key Words: starch digestion

183 Effects of different weaning strategies when feeding moderate and high milk replacer rates on ruminal bacteria taxonomic profile, diversity, and community structure in Holstein calves.

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Ruminal bacteria profile can be greatly affected by pre-weaning diet and the strategy (STEP) used to wean calves. A 2 × 2 factorial design [moderate (MOD) or high (HI) milk replacer (MR) feeding rates and one step (ONE) or gradual (GR) weaning] was utilized to study how pre-weaning programs affect ruminal bacteria diversity from 2 to 4 mo of age. Calves ($n = 50$, 56 d of age) were randomly assigned to 1 of the 4 pre-weaning treatments. The initial BW for HI and MOD calves was 78.9 and 74.3 ± 1.92 (kg). Bacterial diversity was determined using the 16S rRNA gene (V4 region) for 0, 14, 28, 42, 56 d after weaning. The statistical model included the fixed effects of MR, STEP, DAY and their interactions and the random effect of calf nested within treatment. Differences were declared at $P \leq 0.05$. At MOD feeding, *Prevotellaceae* relative abundance (rA, %) was reduced for GR vs. ONE weaning (48.8 vs. 55.4 ± 2.1%) only at MOD feeding. For GR weaning, a reduction was also observed in MOD compared with HI feeding. Furthermore, MOD feeding reduced the rA of *Succinivibrionaceae* vs. HI feeding (3.8 vs. 5.5 ± 0.64%). At HI feeding, ONE weaning reduced *Veillonellaceae* rA compared with GR (5.4 vs. 7.5 ± 0.8%) but no differences were observed within MOD (6.2%). As calves went from 0 to 56 d postweaning, *Lachnospiraceae* rA was decreased for 56 vs. 0 to 42 d (3.2 vs. ~11.8 ± 1.29%); *Succinivibrionaceae* was increased for 56 vs. 14 to 42 d (22.1 vs. ~2.37 ± 1.88); *Veillonellaceae* was higher for d 14 compared with 28, 42, and 56 d (8.4 vs. 4.8, 5.7, and 5.8 ± 0.77); and unidentified *Clostridiales* was reduced for d 56 vs. 0 to 42 d (1.30 vs. ~3.27 ± 0.26). The phylogenetic diversity (richness) was higher for d 42 (15.1 vs. 12.8 for the rest) and 56 vs. 0 and 14 d (13.8 vs. 11.9 ± 0.38). Concurrently, a PERMANOVA analysis of weighted UNIFRAC distances indicated a difference in community structure across periods and treatments ($P < 0.01$). In conclusion, both MR feeding rate and weaning strategy influenced ruminal bacterial rA independent of time after weaning; nonetheless had no effect on richness.

Key Words: calves, post-weaning, microbiome

184 Effect of fat-embedded calcium gluconate on lactation performance and metabolism in dairy cattle.

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Butyrate and its fermentative precursors have demonstrated multiple beneficial effects to the gastrointestinal morphology and function, such as

the stimulation of epithelial cell proliferation, improvement of gut barrier function and support of the general ecological and physiological homeostasis. It has been proposed that by improving gut integrity and function, less energy is partitioned toward immune responses related to xenobiotic infiltration, allowing more energy to be available for productive purposes. Gluconic acid and its salts have previously been shown to have a prebiotic effect in the lower gut of monogastric animals where it serves as a precursor for butyrate, though work in ruminants is limited. The objective of this study was to evaluate the efficacy of supplementing calcium gluconate embedded in a hydrogenated fat matrix on milk production, milk fatty acid composition and hindgut VFA profile in lactating dairy cattle. Forty-five lactating Holstein cows were used in a 3 × 3 Latin square consisting of three 28-d periods during which animals were offered a basal ration supplemented with 3 different compound feeds: a negative control in mash form containing no gluconate, or the same mash feed or a pelleted form, both containing calcium gluconate supplement at a targeted rate of 16 g/d. Data were analyzed using a mixed model treating animal as a random effect and treatment, block, period and parity as fixed effects. Treatments resulted in increases in yields of milk fat ($P = 0.03$) and fat-corrected milk ($P = 0.02$), as well as changes in milk fatty acid composition, which were similar to those seen in response to post-ruminal butyrate supplementation and suggested increases in both de novo milk fatty acid synthesis and incorporation of extra-mammary fatty acids. Changes in concentrations of plasma BHB and NEFA also supported this hypothesis. No differences were observed in fecal concentrations of butyrate. Future work to characterize the eventual effects in the gut lumen, as well as changes in the structure and function of the hindgut epithelium in response to fat-embedded calcium gluconate supplementation is warranted.

Key Words: calcium gluconate, butyrate, dairy cattle

185 The effects of adding exogenous amylases, a protease and their combinations on in vitro dry matter and starch digestibility of mature dent corn grain.

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The objective was to evaluate 4 amylases, a protease and their combinations on ruminal in vitro dry matter (IVDMD) and starch digestibility (IVSD) of mature dent corn grain using a batch culture system. Treatments were control (no enzyme), 4 amylases (4Cs4, 5Trga, 6Afuga, 7Fvga; 0.25 mg/g DM each), a protease (11P14L) and their respective combinations. Experimental design was a randomized complete block and treatments were arranged in a 2 × 5 factorial design with 2 levels of 11P14L (0, 0.25 mg/g DM) and 5 levels of amylase (0 or 0.25 mg/g DM each of 4Cs4, 5Trga, 6Afuga, and 7Fvga) resulting in 10 treatments incubated for several time points (0, 3, 7, 9, 12, and 24 h) in quadruplicates in 3 independent runs (blocks). Mature ground (4-mm) dent corn grain was used as substrate (0.5 g) and enzymes were directly applied on the substrate before incubating in pooled rumen fluid collected from 3 rumen-cannulated lactating dairy cows. Data were analyzed using GLIMMIX procedure of SAS to test for main effects of amylase, protease and the interaction. Block (run) was used as random factor. Synergistic effects of individual amylase × protease within each time point were tested through contrasts (4Cs4 vs 4Cs4 × 11P14L; 5Trga vs 5Trga × 11P14L; 6Afuga vs 6Afuga × 11P14L; 7Fvga vs 7Fvga × 11P14L). Main effects of amylase and protease on IVDMD were observed at all time points ($P < 0.01$). Synergistic improvements in IVDMD were observed with every combination of amylase and protease after 3, 7, 9, 12, and 24h of incubation ($P < 0.05$). Based on the contrast values, synergistic improvement in IVSD was observed with the combination of 5Trga × 11P14L and 6Afuga × 11P14L at 3, 7 and 12 h of incubation ($P < 0.05$). Similarly, the combination of 4Cs4 × 11P14L and 7Fvga × 11P14L resulted in IVSD improvement at 12 and 3 h of incubation, respectively ($P < 0.05$). In conclusion, IVDMD and IVSD were increased by exogenous enzymes with greater improvements observed with the combination of amylases and protease. Future in vivo studies are required to validate these findings and recommend these enzymes for inclusion in livestock feed.

Key Words: starch, amylase, protease

186 Effects of a cashew nut shell extract on production and rumen dynamics in transition dairy cows.

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Objectives were to evaluate the effects of cashew nut shell extract (CNSE, anacardic acid 59% and cardol 18%) on production and rumen dynamics in periparturient Holstein cows. The formulated CNSE final granule contained 50% CNSE. Fifty-one multiparous cows were stratified by previous 305 ME and parity and assigned to 1 of 2 treatments 21 d before expected calving: 1) CON (control diet; n = 17) or 2) CNSE-5.0 (control diet and 5.0 g/d CNSE granule; n = 34). Following parturition, 17 cows from the CNSE-5.0 treatment were reallocated into a third treatment group: CNSE-2.5 (control diet and 2.5 g/d CNSE granule; n = 17) resulting in 3 total treatments postpartum: 1) CON, 2) CNSE-2.5 and 3) CNSE-5.0. Treatments were mixed with ground corn and top-dressed on the base TMR. Effects of treatment, time, and treatment × time were assessed using PROC MIXED. Prepartum DMI was unaffected by treatment, however postpartum it increased linearly with increasing CNSE (19.80, 21.28, and 21.40 kg/d for CON, CNSE-2.5 and 5.0 respectively; $P = 0.01$). Milk yield from cows fed CNSE vs CON tended to be increased (41.33 vs. 38.88 kg/d, $P = 0.06$). CNSE supplementation tended to increase milk protein yield relative to CON (1.42 vs. 1.32 kg, $P = 0.08$). There were no treatment differences detected for ECM, feed efficiency, BW, BCS, energy balance, milk components, MUN or SCC. Prepartum fecal pH decreased in CNSE supplemented cows relative to CON (2%, $P = 0.06$), but no treatment difference was detected for postpartum fecal pH. Rumen pH prepartum was unaltered by treatment. Relative to CON, postpartum rumen pH was increased in CNSE-2.5 and CNSE-5.0 (4.4% and 2.3%, respectively; $P < 0.01$). Dietary CNSE supplementation tended to decrease rumen ammonia nitrogen postpartum relative to CON (34%; $P = 0.10$). Little to no treatment differences were observed in the rumen volatile fatty acid profile prepartum or postpartum. In summary, CNSE supplementation increased postpartum DMI, milk and protein yield, and rumen pH with no changes detected in BW, ECM, or feed efficiency.

Key Words: periparturient, anacardic acid, rumen fermentation

Animal Health: Calves 1

187 Effects of a *Bacillus*-based direct-fed microbial on high- and low-health calf herds.

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Our objectives were to evaluate the effects of a *Bacillus*-based direct-fed microbial designed for calves (DFM, 1.84×10^9 cfu/hd/d, United Animal Health, Sheridan, IN) in commercial dairy herds with historical records of high- (<3% mortality; < 3% scours) and low-heath (>5% mortality; > 5% scours). In Exp. 1, newborn calves received 1 gallon of colostrum within 2h after birth and were transferred to the calf barn. On d 5, calves were moved to group pens where they remained until weaning (d60). Calves received whole milk with (DFM, n = 104) or without any supplement (Control, n = 115) through an automatic calf feeding system (H&L 100, Hold & Laue, Germany). Daily milk intake (DMI) and BWs were recorded individually by the Calf Guide software. Medical cases and treatments (health events) were recorded for each calf and analyzed using PROC GLIMMIX with the main effect of treatment and random effect of pen. Average daily gain (ADG), weight gain (WG) and DMI were analyzed using PROC MIXED of SAS with treatment × pen as main effects. ADG (0.62 ± 0.02 vs. 0.45 ± 0.02 kg) and WG from birth to weaning (36.9 ± 1.24 vs. 27.1 ± 1.24 kg) were higher ($P < 0.0001$) in calves that received DFM compared with Control calves, respectively. DMI and health events did not differ ($P > 0.05$) between treatments. In Exp. 2, newborn calves received colostrum and were transferred to individual hutches. Calves received milk replacer 28/10 with (DFM, n = 236) or without any supplement (Control, n = 212) until weaning (60–90 d old). Health events were recorded individually for each calf and were analyzed using PROC GLIMMIX with the main effect of treatment, and calf ID as random effect. Supplementation of the DFM from birth to weaning resulted in a 10.3% decrease ($P = 0.0494$) in health events (DFM 44.9% vs. Control 55.2%). Digestive (scours with or without blood and constipation) cases ($P = 0.0639$) and percent of treated calves ($P = 0.1443$) tended to decrease with the supplementation of the DFM (33.5% vs. 42.9%, and 44.5% vs. 51.9% for DFM vs. Control, respectively). Supplementation of a *Bacillus*-based DFM to calves from birth to weaning increased performance and decreased health events.

Key Words: direct-fed microbial (DFM), calf, performance

188 Feeding *Saccharomyces cerevisiae* fermentation products modulates immune function and reduces the severity of bovine respiratory syncytial virus infection in preweaned calves.

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Bovine respiratory disease (BRD) is a leading cause of mortality in preweaned dairy calves and weaned dairy heifers. Given increasing concerns related to the development of antimicrobial resistance, there is interest in identifying alternative strategies which can be used to reduce the impact of BRD. The objective of this study was to determine the effects of oral supplementation with *Saccharomyces cerevisiae* fermentation products (SCFP; SmartCare and NutriTek; Diamond V) on the outcome of bovine respiratory syncytial virus (BRSV) infection in preweaned dairy calves. Twenty-four, 1–2 d old calves were assigned 2 groups (12/group): 1) control, base milk replacer and calf starter; or 2) SCFP treated, milk replacer with 1 g/d SmartCare and calf starter top-dressed with 5 g/d NutriTek. Calves were challenged with ~10^4 (Median Tissue Culture Infectious Dose, $TCID_{50}$) BRSV on d 21. Calves were monitored for clinical signs. Nasal swabs were collected to monitor virus shedding. Peripheral blood and bronchoalveolar lavage (BAL) samples were collected for immunologic analyses. Calves were euthanized on d 10 post infection to evaluate gross lung pathology and pathogen load in the lung tissue. Following BRSV infection, calves receiving SCFP had significantly reduced clinical disease scores compared with controls ($P = 0.030$); reduced airway neutrophil recruitment ($P = 0.002$) and reduced lung pathology ($P = 0.031$). Calves receiving SCFP shed less virus than control calves ($P = 0.028$) and tended toward lower viral loads in the lungs ($P = 0.051$). Immune cells from the peripheral blood of SCFP treated calves produced increased ($P < 0.05$) quantities of IL-6 and TNFα in response to toll-like receptor stimulation; while cells from the BAL secreted less ($P < 0.05$) proinflammatory cytokines in response to the same stimuli. Results from this study suggest that supplementing with SCFP modulates both systemic and mucosal immune responses and may improve the outcome of an acute respiratory viral infection in preweaned dairy calves.

Key Words: *Saccharomyces cerevisiae* fermentation products, bovine respiratory disease, innate immunity

189 Assessing the utility of leukocyte differential cell counts for predicting morbidity, mortality and growth in a grain-fed veal facility: A prospective single cohort study.

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On farm machine leukocyte differential cell counts (DCC) that can be acquired quickly may be useful to augment calf risk identification protocols at the time of arrival at a veal or dairy beef operation. The objective of this study was to assess the utility of DCC taken at the time of arrival at a grain-fed veal facility and 72 h post arrival for determining morbidity risk, mortality risk and growth during the production cycle. Data were collected between June and October 2018, from 240 calves upon arrival and a subset of 160 calves 72 h post arrival at a veal research facility in Ontario, Canada. DCC were evaluated using the QScout BLD test for leukocyte differential cell counts (Advanced Animal Diagnostic, Morrisville, NC). All calves were screened using a standardized protocol and a blood sample was collected to evaluate serum total protein (TP) and DCC. Cox proportional hazards models were constructed for both morbidity and mortality outcomes. Mixed linear regression models were constructed for the outcome of average daily gain. Models were constructed in Stata 15 (StataCorp LP, College Station, TX). Results from data collected at the time of arrival suggest that TP values >5.1 g/dL reduce the hazard of mortality (HR = 0.29, $P < 0.001$) and a rectal temperature >39.6°C was associated with an increased hazard of morbidity (HR = 1.48, $P = 0.04$). Calves that were dehydrated gained less (−0.09 kg/d, $P = 0.03$), however, an increased lymphocyte count was associated with calves having a higher level of growth (+0.05 kg/d, $P = 0.02$). Results from DCC collected 72 h post arrival suggest that lymphocyte counts between 4.8 and 5.8×10^9 cells/L decrease the hazard of mortality (HR = 0.22, $P = 0.03$) and $>7.0 \times 10^9$ cells/L decrease the hazard of morbidity (HR = 0.56, $P = 0.02$), whereas, neutrophil counts $>6.0 \times 10^9$ cells/L increased the hazard of mortality (HR = 5.2, $P = 0.02$). This study demonstrates that machine DCC at the time of arrival and 72 h post-arrival has potential for use in calf risk identification protocols in veal and dairy beef facilities.

Key Words: male dairy calf, biomarker, risk factors

190 Effect of colostrum replacer to ameliorate a disease bout in preweaned calves on an automated feeder.

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Changes in feeding behavior monitored through an automated milk feeder (AMF) have been associated with disease development. The objective of this study was to determine if an intervention with 1 dose (375g) of colostrum replacer (CR; PreMolac Plus, Zinpro, MN) or a placebo milk replacer (28% CP 20% fat, Cows Match, Land O Lakes, MN) fed across 3 d (1L 125g/d) to triggered alarm calves (n = 110 alarms; n = 57 placeb0, n = 53 CR) could lower the likelihood of a disease bout occurring (ameliorate) before weaning. An algorithm was set to trigger an alarm when negative deviations of milk intake or drinking speed were detected in relation to a baseline average of 12 d. Calves (42 placebo;42 CR) were enrolled on the AMF at age 4.0 ± 2.0 d (mean ± sd), were offered 10 L/d milk replacer and had ad libitum access to starter measured by automated feeders. Calves were scored daily for bovine respiratory disease (BRD) and diarrhea and weighed and scored using a lung ultrasound 2x weekly. A BRD score >4 and an area of consolidated lung >2.9 cm^2 was considered a BRD bout. Treatments were not different on the d before alarm by health status, weights, and ages, alarms were triggered at average 32.1 ± 12.5 d of age (mean ± sd). The effect of CR to ameliorate BRD and diarrhea were calculated with logistic models using SAS (9.4), time was a fixed effect, with milk intake as a co-variate. The effect of CR on ADG was determined using a linear mixed model with dam parity and BRD status as co-variates, repeated by d and calf, with birth date as a random effect. Post-intervention, placebo calves had a 1.64 (95% CI:1.10–2.43) times greater odds of having a BRD bout compared with CR calves for the 7-d following intervention. Moreover, placebo calves had a 1.50 (95% CI: 1.10–2.1) times greater odds of having lung consolidation than CR calves. No difference was found in the likelihood of diarrhea (OR 0.91; 95% CI: 0.73–1.12) or ADG (placebo 0.73 ± 0.07 kg/d) and CR (0.70 ± 0.08 kg/d) between interventions. Results suggest CR may reduce the likelihood of a BRD bout in calves, but not influence growth. Future research should examine which properties of CR ameliorate BRD bouts.

Key Words: bovine respiratory disease, performance, supplement

191 Effects of different blood buffers administered in electrolyte solution to grain-fed veal calves experiencing diarrhea.

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The objective of this randomized controlled trial was to examine differences between treating male dairy calves experiencing diarrhea with either a basic electrolyte powder (BBP) composed of sodium bicarbonate (50.7 mmol/L), a mixed buffer powder (MBP) including sodium bicarbonate (33.8 mmol/L), sodium citrate (8.4 mmol/L), sodium acetate (6.3 mmol/L) and potassium citrate (1.9 mmol/L), or a liquid electrolyte composed of sodium acetate (50.1 mmol/L) (LSA). All 3 electrolyte solutions provided 50 mmol/L of blood buffers and a similar strong ion difference. Holstein male calves sourced from auction barns and local dairies were delivered in one batch to the research site. Calves were housed in individual pens and fed a 24% CP and 17% fat milk replacer (MR) twice daily. Starter grain and water were offered ad libitum. A total of 45 calves were randomly enrolled in 1 of the 3 treatments (MBP: n = 14; HAL: n = 16; BBP: n = 15) when experiencing either 2 consecutive days of a fecal score 2 (runny, spreads easily) or 1 day with a fecal score of 3 (liquid void of solid material). Calves were blocked by the different enrollment criteria. The respective electrolyte was administered via esophageal tube one hour after feeding MR until the fecal score returned to a normal or pasty consistency. Blood gas measures were taken at 1, 8, and 24 h post the initial electrolyte feeding and weight was measured at 1, 2, 7, 14, and 28 d post-enrollment. Mixed repeated measures linear regression models were built using STATA 14 to assess the impact that the electrolyte had on the blood gas parameters and BW. Statistical significance was defined as $P < 0.05$. As compared with BBP, MBP significantly increased blood CO2 at 8 and 24 h, bicarbonate at 24 h, base excess at 8 and 24 h, and anion gap at 24 h, all indicative of improved blood pH. Feeding LSA noted similar results to MBP. However, LSA had a significantly higher BW on d 2 as compared with MBP. Although a severe dehydration challenge was not present, MBP improved acid-base status of calves compared with BBP, whereas LSA performed similarly to MBP.

Key Words: calf, veal, electrolyte

192 Production and bioactivity of anti-*Streptococcus equinus* antibodies.

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Organisms previously classified as *Streptococcus bovis* (i.e., *S. equinus*) are common in the rumen and feces and have been described as commensal bacteria in humans and animals. They may act as opportunistic pathogens contributing to ruminal acidosis and mastitis. Any microorganism that colonizes the rumen must possess the capability of adherence to the ruminal surface for multiplication. The adhesins responsible for bacterial adhesion are generally targets of antibodies that can inhibit attachment. In this study we evaluated the production and bioactivity of antibodies against *S. equinus*. Twenty-four hens allocated to 8 cages were divided in 2 groups: one group was immunized with the control vaccine (adjuvant plus phosphate-buffered saline) and the other was administered the test vaccine containing *S. equinus* strain JB1 as the antigen. The IgY concentrations in egg yolks were determined with Chicken IgY ELISA Kit Fine Test and analyzed by the unpaired *t*-test. IgY bioactivity was evaluated by plate trapped antigens-enzyme linked immuno sorbent assay (PTA-ELISA). In the PTA-ELISA 5.3×10^8 colony-forming units/mL of *S. equinus* were pre-coated onto the wells of a multi well-plate for both control and sample testing. These wells were incubated with serially diluted anti-*S. equinus* antibody and then with the second antibody-enzyme conjugate followed by the chromogenic enzyme substrate in duplicate. The control and test production were 22.5 and 104.1 mg/yolk of IgY, respectively. The PTA-ELISA revealed high yield bioactivity of IgY against *S. equinus*, and the 1:16 dilution was selected for greater formation of antibody-antigen complexes. Both antibody affinity and the accessibility of epitopes on the plate are critical factors in determining whether the diluted antibody levels exceed the threshold required for complex formation. These results provide a good approximation of the optimal relationship between antigen and antibody, guiding the dosages in further animal tests.

Key Words: antibody-antigen complex, immunization, microbiome

Breeding and Genetics

193 Association of a SNP in the *DGAT1* gene with productive and reproductive performance and profitability in grazing cows milked once and twice a day. N. Lopez-Villalobos*[1], H. B. P. C. Ariyarathne[1], K. Gedye[2], M. Correa-Luna[1], and D. J. Donaghy[1], [1]*School of Agriculture and Environment, Massey University*, *Palmerston North, New Zealand*, [2]*School of Veterinary Sciences, Massey University*, *Palmerston North, New Zealand*.

A single nucleotide polymorphism (SNP; rs109421300) of the diacylglycerol acyltransferase 1 (*DGAT1*, BTA 14) gene has been associated with fat and protein percentages in dairy cows milked twice a day (TAD). This study evaluated the association between rs109421300 genotype with productive and reproductive performance and profitability in grazing cows milked TAD and once a day (OAD) at Massey University, New Zealand. From July 2017 to May 2018, 161 cows (32 TT, 65 CT and 64 CC genotypes) were milked OAD and 485 cows (91 TT, 254 CT and 140 CC genotypes) were milked TAD. Yields of milk (MY), fat (FY), protein (PY), along with fat percentage (FP), protein percentage (PP), BW, and different fertility measures from the 646 cows in the 2 herds were analyzed with a linear model that included the fixed effects of milking frequency (MF), lactation number (LN), SNP genotype, interactions (MFxSNP, LNxSNP) and covariates for the linear effects of proportion of Holstein-Friesian, heterosis Holstein-Friesian x Jersey, and deviation from herd median calving date. DM intake was derived from estimations of metabolizable energy requirements. Milk income minus feed cost (MImCF) was expressed per tonne of predicted DM intake. Milk income was based on the milk payment system; \$7.141 × kg FY + \$6.156 × kg PY - \$0.033 × kg MY. Compared with TT cows, the CC cows had significantly higher FP, PP and FY and lower MY in both milking frequencies. The CT cows had values between the TT and CC cows. The combined changes of milk composition, yields and BW resulted in CC cows with the highest MImFC per t DM (\$344 ± 5.4), followed by CT (\$328 ± 5.1) and TT (\$316 ± 7.0) cows in the OAD farm. The respective values in the TAD farm were \$381 ± 4.2, \$365 ± 3.2 and \$348 ± 4.7. The allele substitution effect for MImFC was not significantly different between MF. The effects of SNP genotype on the other productive and reproductive traits were not significant. Selecting cows for the C allele of the SNP rs109421300 can be beneficial for New Zealand dairy farmers.

Key Words: milking frequency, SNP genotype, *DGAT1*

194 Direct, indirect, and pleiotropic genetic effects associated with calving ease, retained placenta and metritis in US Holstein cows. R. A. Teixeira*[1,2], L. T. Dias[1,2], A. Sigdel[1], and F. Peñagaricano[1], [1]*University of Florida*, *Gainesville, FL*, [2]*Universidade Federal do Paraná*, *Curitiba, PR, Brazil*.

Calving ease, retained placenta and metritis are important traits that directly impact animal welfare, productivity, and farm profitability. These traits are highly correlated, and our goal was to reveal genomic regions with direct and indirect genetic effects. We hypothesized that calving ease affects both retained placenta and the incidence of metritis, while retained placenta has also a direct effect on metritis. Direct, indirect, and pleiotropic genetic effects were detected using both marginal and conditional whole-genome scans. Data consisted of about 28k health records on 13k Holstein cows across the first 6 lactations. Genotype data for 58k SNP markers were available for 6.1k cows with phenotypic records and 1.4k sires in the pedigree. The ssGBLUP method was used to identify genomic regions and individual genes that explain more than 0.5% of the additive genetic variance. Whole-genome scan detected regions on BTA6, BTA11 and BTA17 with direct effects on calving ease. These regions harbor several genes including *FAHD2A* and *NPHP1*. Genomic regions on BTA2, BTA10, BTA19, BTA23, BTA25 and BTA29 were found to have direct effects on retained placenta. These regions harbor several genes, including *ZNF690* and *SNX22*, which are involved in caruncle-cotyledon separation. Genomic regions on BTA5, BTA8, BTA12, BTA19 and BTA29 were found to have direct effects on metritis. These regions contain putative candidate genes, such as *RBFOX2, MYH9, CCL5, CCL14* and *CCL16*, which are involved in immunity. Genomic regions having pleiotropic effects were found on BTA14 for calving ease and metritis, and on BTA15 for retained placenta and metritis. Moreover, indirect effects were detected on BTA20 for retained placenta, and on BTA13 for metritis. Overall, our research has multiple benefits, including better understanding of the biology underlying these correlated phenotypes, promote the development of new therapies and mitigation strategies, and contribute to the design of novel breeding strategies using genomic information.

Key Words: dystocia, postpartum uterine disease, structural model

195 Estimation of genetic parameters for dry matter intake, energy-corrected milk, metabolic body weight, and milk yield using a random regression model. K. Houlahan*[1], C. F. Baes[1,2], F. Miglior[1], G. A. Oliveira Jr.[1], F. S. Schenkel[1], and T. C. S. Chud[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph*, *Guelph, ON, Canada*, [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern*, *Bern, Switzerland*.

Feed efficiency has been heavily researched in recent years. As feed costs rise and the environmental impacts of agriculture become more apparent, improving the efficiency with which dairy cows convert feed to milk is increasingly important. However, feed intake is expensive to accurately measure on large populations, making the inclusion of feed efficiency into breeding programs difficult. Through technological advancements, accurate measurements on traits related to feed efficiency, such as dry matter intake, BW, body condition score, and milk components have become more readily available. Understanding how the genetic parameters of traits related to feed efficiency vary throughout a lactation curve is valuable. In this study, 75,255 daily feed intake records, 16,786 milk production records, and 30,615 weekly BW records were collected on 610, 827, and 331 Canadian first lactation cows, respectively, from 2007 to 2019. Genetic parameters were calculated for these 3 traits by week of lactation using a random regression model. Heritability estimates for all traits were lower in the first stage of lactation compared with the later stages of lactation. The results of this study contributed a better understanding of the change in genetic parameters across the first lactation, providing insight on potential selection strategies to include feed efficiency into breeding programs.

Key Words: dairy cattle, feed efficiency, genetics

196 Genetic parameters of passive transfer in Holstein calves. I. Haagen*[1], L. Hardie[1], B. Heins[2], and C. Dechow[1], [1]*The Pennsylvania State University*, *University Park, PA*, [2]*University of Minnesota*, *Morris, MN*.

The objectives of this study were to estimate genetic parameters for calf serum total protein (STP) and failure of passive transfer (FPT) and to calculate approximate genetic correlations between STP and commonly evaluated fitness and health traits. STP measurements were extracted from farm management software and available for 7518 organic Holstein calves born between July 2013 and June 2018 on 2 organic dairies. FPT was defined as calves with STP ≤5.2 g/dL and coded as a binary trait. STP and FPT variance components were estimated using single-trait linear and threshold models, respectively. In addition, a 2-trait linear animal model was used to estimate the genetic correlation between STP and FPT. All models included the fixed effects of age at measurement (1, 2, or 3 d), age of dam (2, 3, 4, 5, or ≥6 years), and herd-year-month of birth (levels = 98); random effects were birthdate within herd and direct additive genetic. Approximate genetic correlations of STP with fitness and health traits were estimated using predicted transmitting abilities from 256 proven sires with at least 10 daughters with STP measurements. In total, there were 18,566 animals in the pedigree. The heritability of STP was 0.08 ± 0.02, the heritability of FPT was 0.06 ± 0.04, and the genetic correlation between STP and FPT was unity. The approximate genetic correlations of STP with productive life and livability were 0.40 ± 0.05 and 0.42 ± 0.05, respectively. STP exhibited negative and favorable genetic correlations with sire stillbirth, daughter and sire calving ease (range −0.23 to −0.31). Further, the genetic correlation between STP and gestation length was −0.30 ± 0.07, and the genetic correlation between STP and early first calving was 0.25 ± 0.05. These results suggest that calf STP is heritable and genetically correlated with cow longevity and calving traits.

Key Words: calf serum total protein, heritability

198 Assessment of methane emission traits in Canadian Holstein cows. S. Kamalanathan*[1], T. C. S. Chud[1], D. Hailemariam[2], P. Stothard[2], G. Plastow[2], F. Miglior[1], C. F. Baes[1,3], and F. S. Schenkel[1], [1]*Center for Genetic Improvement of Livestock, University of Guelph*, *Guelph, ON, Canada*, [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta*, *Edmonton, AB, Canada*, [3]*Institute of Genetics, Vetsuisse Faculty, University of Bern*, *Bern, Switzerland*.

The reduction of greenhouse gas emissions has become an important area of research as Canada aims to reduce methane (CH_4) emissions. This puts pressure on the Canadian dairy industry, as dairy cattle are responsible for roughly 13% of the total agricultural sector's CH_4 emissions. In ruminants, CH_4 is an output of the enteric fermentation produced by methanogens belonging to the archaea domain of microbes in the rumen. High enteric CH_4 emissions of dairy cattle also cause a decrease in productivity due to a loss of gross energy intake. Breeding objectives should focus on improving the overall sustainability of dairy cattle by reducing CH_4 emissions without negatively affecting economically important traits. The aim of this study is to determine the optimal methane trait for use in dairy cattle breeding and to investigate the genetic architecture of methane emission and its correlation with feed efficiency using Canadian data. A total of 2,451 daily CH_4 production (grams/day) records from 335 Holstein cows were recorded from Canadian research herds located in Ontario (from 2017 to 2019) and Alberta (from 2015 to 2018) using GreenFeed stations (C-Lock Inc., Rapid City, South Dakota, USA). The overall mean and respective standard deviation (SD) of CH_4 was 411.4 (116.6) g/d with a coefficient of variation of 28.3%. Higher mean of CH_4 (476.3g/d) with a SD of 97.5 was observed for the Ontario herd compared with the Alberta herd (351.3 g/d) with an SD of 99.4. In contrast, the Alberta herd showed a higher coefficient of variation (28.3%) than the Ontario herd (20.5%). This result could be due to the differences in the number of times CH_4 was collected in the herds. The need and alternative ways to standardize CH_4 emissions between herds will be also investigated. The results of this study will help identify the most appropriate methane trait when monitoring/selecting for lower methane emissions in dairy cattle and will reveal the level of genetic correlation between methane emission and feed efficiency in Canadian Holstein cows.

Key Words: CH_4, breeding, methane emission traits

Dairy Foods: Dairy Products

199 Effect of soy lecithin concentration on the formation and stability of ultrasound emulsions. C. K. Nyuydze*, J. Reineke, and S. I. Martinez-Monteagudo, *South Dakota State University*, *Brookings, SD*.

Emulsification is a key processing step during the manufacture of dairy beverages. Inadequate emulsification negatively impacts the shelf-life of beverage due to the development of coalescence, flocculation and creaming.Soy lecithin is commonly used to stabilize food emulsions.Recently, consumers identify soy lecithin as an undesirable ingredient, and beverage processors are actively exploring alternatives for soy lecithin.This study focuses on evaluating the effect of lecithin on the stability of emulsions prepared by ultrasonication.Emulsions were manufactured by mixing whey protein concentrate(8%),maltodextrin and sucrose(25%)vegetableoil(4%)and soy lecithin added at different concentrations(0,3and6%). The mixture was emulsified by ultrasonication using an acoustic intensity of either 342.5 ± 5.8, 443.6 ± 7.5, or 524 ± 8.9 kJ $m^{-2}s^{-1}$.After emulsification, the beverages were stored at 4°C, and the stability was evaluated weekly during 3 weeks.The stability of the ultrasound emulsions was evaluated in terms of particle size, gel electrophoresis, rheological behavior and confocal laser scanning microscope.Overall, the application of ultrasound results in a bimodal distribution of particle ranging from 310 to 748 nm.The lowest range of distribution (270 ± 10.8nm)was observed in those emulsions treated at the highest intensity(524 ± 8.9 kJm^{-2} s^{-1}).Interestingly, the mean particle size of those samples without lecithin increased during storage from 520 to 750 nm.CLSM images indicated aggregation of proteins during storage as well as larger oil droplet on samples treated at 342.5 ± 5.8 kJ $m^{-2}s^{-1}$ and 0% lecithin concentration.The rheological analysis revealed that all samples displayed a distinctive viscoelastic region.The linear segment was significantly($P < 0.05$)longer in the emulsion formulated with soy lecithin(6%) and treated at 524 ± 8.9 kJ m^{-2} s^{-1}.In general, the combined effect of soy lecithin and ultrasonication yielded a stronger gel and shear-thinning behavior.The application of ultrasound seems to functionalize whey protein-based emulsifiers via processing and compositional parameters that enhance interactions between ingredients.

Key Words: ultrasound, lecithin, emulsion

200 Multi-origin skim milk powder comparative benchmarking. D. E. Otter[1], C. T. Tay[2], R. B. Hashwam[2], K. G. Lim[2], M. B. Roque[2], H. Hardigaluh[3], and A. Bienvenue*[4], [1]*DEO Dairy Consulting*, *New Zealand*, [2]*Food Innovation & Resource Centre, Singapore Polytechnic*, *Singapore*, [3]*U.S. Dairy Export Council Singapore Ltd*, *Singapore*, [4]*U.S. Dairy Export Council*, *Arlington, VA*.

What makes a skim milk powder (SMP) suitable for use in recombined ultra-high-temperature (UHT) milk and/or evaporated milk (EM) applications? Twenty SMP samples manufactured in Canada, Europe, New Zealand and the United States, and intended for UHT or EM use in Southeast Asia, were characterized and used in EM and UHT milk model systems that were tested over 6 mo of storage. The goal was to compare the performance of various origin SMP. Compositionally all the SMP samples met CODEX requirements. Some manufacturers standardized protein levels, while others did not. SMP protein ranged from 32.7 to 37.4% m/m (as is), moisture 3.5 to 4.4% and fat 0.6 to 1.5%. There was minimal variation across the samples in pH, titratable acidity, solubility index, viscosity, bulk density, scorched particles, hygroscopicity, and water activity. Whey protein nitrogen index (WPNI) values were indicative of heat treatment type. Heat coagulation time (HCT) at 140°C varied greatly between the samples (6.5 to 31.6 min). Correlation between HCT and WPNI was 0.57; so WPNI does not appear to be a good predictor of heat stability. The SMPs' color was similar, although there was slightly more yellow (b*) in the powders from NZ. The microbial analysis revealed supplier-specific (not origin) differences between the samples. Aerobic spores (S) were measured using a heat shock of 80°C/12min and heat resistant spores (HRS) 100°C/30min. Mesophilic S and HRS were low. Large variations were seen in thermophilic (T) aerobic plate counts, S and HRS; 11 and 16 SMP samples (EU, NZ, US origin) had low T-S and T-HRS < 300 cfu/g respectively. In the UHT application, all samples withstood the heat treatment without gelling or excessive browning. After storage, minor differences were observed between the samples. In the EM application, one sample gelled after heating, and varying degrees of sedimentation were observed after processing and during storage. Six EM samples (CA, NZ, US origin) performed well throughout the 6 mo shelf life, exhibiting only minor phase separation. Our results show that different origin SMP samples were similar across many parameters with differences in performance being supplier rather than country-specific.

Key Words: skim milk powder, UHT

201 Electrical resistance tomography for monitoring the rehydration characteristics of high-protein dairy powders. K. S. Babu* and J. Amamcharla, *Kansas State University*, *Manhattan, KS*.

Electrical resistance tomography (ERT) is a novel, robust, and low-cost method offering non-invasive and remote sensing. The objective of this study was to develop a method to characterize the rehydration behavior of milk protein concentrate (MPC) powders using ERT in 2 configurations (circular sensor and linear probe). To validate the method, 2 lots of fresh MPC85 and MPC90 powders were procured from a commercial manufacturer. Focus beam reflectance measurement (FBRM) was used as a reference method to follow rehydration characteristics. To evaluate the rehydration characteristics, MPC powders were reconstituted to 5% (wt/wt) total solids at room temperature and the rehydration characteristics of MPC powders were monitored for 30 min using ERT. MPC dissolution was characterized in terms of overall mean conductivity, the area under the mean conductivity curve, and equilibrium dissolution time from the ERT data. Particle count changes from FBRM revealed that MPC90 had more resistance to dispersing in water. As the dissolution time proceeded, mineral ions and proteins are released and consequently increased the overall conductivity as observed by both the sensing planes. Both the configurations were able to effectively capture the differences in the dissolution of MPC85 and MPC90 powders. As the protein content increased, the particle dispersion rate decreased and, consequently, a decrease in mean conductivity and reduction in the area were observed, indicating a reduction in solubility. For the circular sensor configuration, MPC85 and MPC90 showed the maximum conductivity of 0.20 and 0.16ms/cm, respectively. Whereas, for the linear probe configuration MPC85 and MPC90 showed the maximum conductivity of 0.16 and 0.13ms/cm, respectively. Both the configurations showed a significant difference ($P < 0.05$) in the maximum conductivity of MPC85 and MPC90. Overall, the ERT can be a robust and low-cost method to characterize the dissolution behavior of high-protein dairy powders.

Key Words: electrical resistance tomography, milk protein concentrate powders, rehydration

202 Investigating the suitability of acid whey in the manufacture of cornichon pickles. O. Ozturk, S. Cebeci, O. Yemis, A. C. Mehmetoglu, and M. Ozturk*, *Sakarya University, Food Engineering Department*, *Sakarya, Turkey*.

The production of Greek-style yogurt has dramatically increased in recent decades worldwide, which lead to increased production of acid whey. Unlike whey, acid whey still has little or no economic value and handling acid whey is still a problem for the Greek yogurt manufacturers. In this study, suitability of acid whey in the production of cornichon pickle production was investigated. Pickles were manufactured with fermentation (F) and pasteurization (P) methods with either acid whey (A) or vinegar

(V) (as control). Six batches of pickles were manufactured over 2 mo period. 660cc jars (13.8 × 86.7cm) were used for pickling (~300g of pickles), and for each manufacture 14 jars of FA, 14 jars of FV, 10 jars of PA and 10 jars of PV pickles were manufactured. Brines were prepared with 1% titratable acidity, and fermented pickles and pasteurized pickles were manufactured with 8 and 6% salt, respectively. Changes in the textural, microbial, visual and sensory properties were monitored for 3 mo of storage. For each time point, 1 jar of pickles were used for compositional and textural analyses and 1 jar is used for sensory panel. Puncture stress and endocarp hardness of pickles were monitored using texture analyzer. The sensory properties (acid, bitter, salt) of pickles were analyzed by using quantitative descriptive analyses (15 point scale) with trained sensory panelists. For the pickles manufactured either with fermentation or pasteurization, using acid whey or vinegar as brine did not influence the texture and color during storage. Pickle pH was unaffected by the brine used for fermented pickles, but PA pickles exhibited significantly higher pH values (~4.6) compared with PV pickles (~4.0). No significant differences were observed in acidity, bitterness and saltines between FA and FV pickles during storage; however, PA pickles exhibited slight (<1) bitterness in the first 1 mo of storage. Using acid whey as brine increased the development of lactococci species and lactococci numbers were higher (1.5–2 log) in pickles manufactured with acid whey during storage for both fermented and pasteurized pickles. In all pasteurized pickles, lactobacilli species could not grow independent of the type of brine used. In conclusion, this study shows that acid whey can be a good brine media for pickling.

Key Words: acid whey, pickling

203 The effect of total milk protein, casein, and whey protein ingestion on blood glucose and insulin in rats. T. Olowookere[1], E. Vandenboer[1], Y. Anini[2], M. Akbarifakhrabadi[1], C. Kale[1], N. Tari[3], and B. Luhovyy*[1,2], [1]*Mount Saint Vincent University*, *Halifax, NS, Canada*, [2]*Dalhousie University*, *Halifax, NS, Canada*, [3]*University of Guelph*, *Guelph, ON, Canada*.

High-protein dairy products may reduce postprandial blood glucose and lead to the transient increase of insulin. The ingredients used in high-protein dairy products may include total milk protein (TMP), casein (CN) and whey protein (WP). However, their direct effect on insulin and BG remains unclear. Our objective was to investigate the acute effect of commercial TMP, micellar CN and WP isolates on BG and insulin response in rats. We hypothesized that the magnitude of insulin and BG response would depend on the type of protein. Six male Wistar Han rats with jugular vein catheters and vascular access buttons were used in a randomized crossover. After 6h daylight fasting, each rat was gavaged with 350 mg/3 mL of either TMP, CN, WP or glucose (G) reflecting a human dosage of 10g calculated based on allometric scaling. Each rat received all treatments in random order with a 48h in between. Blood was collected at 0, 15, and 30min for insulin measured with Ultra-Sensitive Rat Insulin ELISA, and at 0, 15, 30, 60, 90, and 120min for glucose measured with HemoCue 201 Glucose Analyzer. The data were analyzed with 2-way RM ANOVA followed by Tukey-Kramer post hoc test. There was an effect of treatment ($P = 0.0002$), time ($P < 0.0001$) and a treatment by time interaction ($P < 0.0001$) on BG over 120min. At 15min TMP, CN and WP, and at 30min CN and WP led to reduced BG compared with G ($P < 0.05$). At 30min, BG was lower after WP compared with CN ($P < 0.05$). There was an effect of treatment on BG area under the curve (AUC) over 120min ($P = 0.001$). All protein treatments resulted in a lower BG AUC compared with G ($P < 0.05$). There was an effect of treatment ($P = 0.02$), time ($P < 0.0001$) and treatment by time interaction ($P < 0.0001$) on insulin over 30min. At 15min, only TMP and CN but not WP led to reduced insulin compared with G ($P < 0.05$). There was an effect of treatment on insulin AUC over 30min ($P = 0.006$). All protein treatments resulted in a lower insulin AUC compared with G ($P < 0.05$). In conclusion, although TMP, CN and WP follow a similar pattern in their effect on BG and insulin, they demonstrate distinct properties at 15 and at 30 min after ingestion.

Key Words: milk proteins, blood glucose, insulin

204 Milk protein fractions in liquid and solid matrices and their sensory perception. E. MacEachern, Y. Xu, P. Kathirvel, and B. Luhovyy*, *Mount Saint Vincent University*, *Halifax, NS, Canada*.

Milk protein fractions (MPF) possess multiple biological activities and can be used in functional dairy products. The current trend among health-conscious consumers is to select products with a lower sugar content and free of artificial sweeteners and flavors. Our objective was to explore the sensory characteristics of MPF isolates solubilized in milk or water, or in a solid matrix without any sweeteners or flavors. We hypothesized that the sensory characteristics of MPF will differ in each matrix. Three randomized, double-blind crossover studies with adults (19–65 y) were conducted. The following 10% (wt/vol) MPF were tested: whey protein isolate by ion exchange (WPI-IE), whey protein isolate by membrane filtration (WPI-MF), glycomacropeptide (GMP), α-lactalbumin (ALA), micellar casein (CN), and milk protein isolate (MPI). In study 1, 40 adults (20 males, 24.4 ± 5.2SD y; 20 females, 22.4 ± 3.5SD y) tested MPF in whole milk. In study 2, 44 adults (22 males, 30.5 ± 9.8SD y; 20 females, 26.6 ± 8.0SD y) tested MPF in water. In study 3, 40 adults (20 males, 26.3 ± 5.3SD y; 20 females, 26.2 ± 5.3SD y) tested freeze-dried whole milk MPF. Acceptability of taste, flavor, mouthfeel, and aftertaste were measured using the 9-point hedonic scale. Sweetness, bitterness, saltiness, astringency was assessed using a 100-mm visual analog scale. Statistical analysis was performed with Friedman's test. In study 1 the mean hedonic scores were between 3.4 and 6.0. There was an effect of treatment on taste, flavor, mouthfeel, aftertaste, sweetness, bitterness, saltiness, and astringency ($P < 0.05$). Taste, flavor, mouthfeel, and aftertaste for ALA was higher compared with MPI, GMP, and CN ($P < 0.05$). In study 2 the mean hedonic scores were between 2.5 and 5.1. Taste and flavor for GMP were lower compared with other treatments except CN ($P < 0.05$). In study 3 the mean hedonic scores were between 3.9 and 4.8, however there was no effect of a treatment on hedonic scores and astringency. WPI-IE, WPI-MF, ALA, and GMP were sweeter than CN and MPI ($P < 0.05$). In conclusion, MPF have different sensory characteristics that can be modulated by a food matrix and potentially improved using small to moderate amounts of natural sweeteners and flavoring agents.

Key Words: milk proteins, hedonic, sensory

Physiology and Endocrinology

205 Methionine and arginine supplementation alters mechanistic target of rapamycin (mTOR) and insulin signaling in bovine subcutaneous adipose explants challenged with C2-ceramide.

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Periparturient cows are exposed to increased circulating levels of ceramide which contributes to insulin resistance. Both Met and Arg promote protein synthesis via the mechanistic target of rapamycin (mTOR) and synthesis of polyamines and glutathione (antioxidants). Our previous work underscored the potential for Met supply to enhance insulin sensitivity in s.c. adipose tissue (SAT). The objective of this study was to investigate effects of enhanced Met and Arg supply alone or in combination on protein abundance of mTOR and insulin signaling pathways in adipose explants during ceramide stimulation. SAT from the tail-head of 4 Holstein cows (parity 4 ± 1.4, DIM 248 ± 38 before slaughter; mean ± SD) was incubated in duplicate for 4 h with one of the following media: ideal profile of essential AA as the control (IPAA; Lys:Met 2.9:1, Lys:Arg 2:1), increased Met (incMet; Lys:Met 2.5:1), increased Arg (incArg; Lys:Arg 1:1), or incMet plus incArg (Lys:Met 2.5:1 Lys:Arg 1:1) with or without exogenous C2:0-ceramide ($100\ \mu M$). Total protein extracted from tissue explants was used for Western blotting. Data were analyzed as a $2 \times 2 \times 2$ factorial using the MIXED procedure of SAS 9.4. There was a triple interaction between Met, Arg and ceramide for phosphorylated (p) protein kinase B (AKT) and p-mTOR ($P < 0.05$). Ceramide stimulation downregulated overall abundance of p-mTOR and p-AKT ($P < 0.05$). Without ceramide stimulation, enhanced Met and Arg alone or in combination led to lower p-mTOR ($P < 0.05$). However, compared with IPAA challenged with ceramide, increased Met or Arg led to greater p-AKT and p-mTOR, with a more pronounced response due to Arg and Met individually ($P < 0.05$). Compared with IPAA challenged with ceramide, enhanced Met or Arg supply resulted in greater activation of mTOR (p-mTOR/total mTOR) and AKT (p-AKT/total AKT), with a more pronounced response due to Arg. Overall, data suggest that greater Met or Arg supply might help preserve SAT functionality during the periparturient period when systemic ceramide concentrations increase.

Key Words: ceramide, amino acid, mechanistic target of rapamycin

206 Branched-chain amino acid supplementation alters protein abundance of mechanistic target of rapamycin (mTOR) and insulin signaling pathway components in bovine subcutaneous adipose explants.

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Branched-chain amino acids (BCAA) are import regulators of mechanistic target of rapamycin (mTOR). In humans and rodents, increased circulating BCAA levels are positively associated with insulin resistance. The objective of this study was to investigate changes in protein abundance of mTOR and insulin signaling pathway components and AA metabolism in bovine s.c. adipose explants in response to increased supply of Leu, Ile or Val. Four Holstein cows (parity 4 ± 1.4, DIM 248 ± 38 before slaughter; mean ± SD) were used in this study. All cows were fed a common lactation diet before slaughter. Subcutaneous adipose tissue (SAT) from the tail-head was incubated with basal media containing an "ideal" mix of essential AA (IPAA; 2.9:1 Lys:Met; 1.8:1 Lys:Thr; 2.38:1 Lys:His; 1.23:1 Lys:Val; 1.45:1 Lys:Ile; 0.85:1 Lys:Leu; 2.08:1 Lys:Arg) or IPAA supplemented with Ile, Val, and Leu to achieve a Lys:Ile of 1.29:1, Lys:Val 1.12:1, or Lys:Leu 0.78:1 for 4 h. Total protein extracted from the explants was used for Western blotting. The statistical model in SAS 9.4 included the fixed effect of Lys:Ile, Lys:Val and Lys:Leu ratios. Compared with IPAA, enhanced Leu and Ile supplementation led to greater activation of protein kinase B (AKT; p-AKT/total AKT) and mTOR (p-mTOR/total mTOR) ($P < 0.05$). However, compared with IPAA, Val supplementation led to lower p-mTOR ($P < 0.05$). Despite protein abundance of solute carrier family 38 member 1 (SLC38A1) and branched-chain ketoacid dehydrogenase kinase (BCKDK) being greater compared with IPAA, supplementation of BCAA also led to greater activation of eukaryotic elongation factor 2 (EEF2; p-EEF2/total EEF2) ($P < 0.05$). Furthermore, compared with increased Leu or Ile, enhanced Val supplementation led to greater abundance of SLC38A1 and BCKDK ($P < 0.05$). Overall, data suggest that enhanced supply of BCAA activates mTOR and insulin signaling in SAT partly due to increased transport into tissue and lower catabolism.

Key Words: branched-chain amino acid, mechanistic target of rapamycin, subcutaneous adipose tissue

207 Body condition alters lipidomic profiles in subcutaneous adipose tissue of Holstein cows during the periparturient period.

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The periparturient period is characterized by excessive adipose tissue (AT) lipid mobilization and increased incidence of metabolic disorders in dairy cows. The aim of this study was to characterize the lipid profiles in s.c. adipose tissue (SAT) between peripartal cows with different prepartal body condition score (HBCS vs. LBCS). SAT from a subset of 10 healthy multiparous Holstein cows from a larger cohort classified as low BCS (LBCS; $3.0 \leq \text{BCS} < 3.25$; n = 5) or high BCS (HBCS; $3.5 \leq \text{BCS} \leq 4.0$; n = 5) at 4 wk prepartum were used. Biopsies obtained on d −15, 7, and 30 relative to parturition were used for lipidomic analysis via liquid chromatography and tandem mass spectrometry. The multivariate statistical analysis of the lipidomic data was performed with the web-based metabolomic data processing tool MetaboAnalyst 4.0. Briefly, as quality control, variables containing more than 50% missing values were not considered for the statistical analysis. The raw data were transformed using the generalized log-transformation and then Pareto scaled to correct for heteroscedasticity. According to the VIP scores using the plots of partial least squares discriminant analysis (PLS-DA) model, there were 15 metabolites with a $\text{VIP} > 2$. Ceramide (d18:1/32:2) and diglyceride (DG) (18:0/22:4) were less abundant in HBCS cows; while those cows had greater triglycerides (TG) (4:0/14:1/16:1; 49:2). Analysis of variance-simultaneous component analysis was performed to ascertain variation in lipid profiles between groups, time and their interaction using MetaboAnalyst 4.0. Sixteen metabolites including 3 ceramides (d18:1/32:2; d50:4; d18:1/18:0), 2 ω-hydroxy fatty acids, 8 DG and 3 TG increased after calving and reached their peaks at d7 postpartum; in contrast, phosphatidylcholine (39:3) decreased after parturition. Time-course profiles of the top 50 lipid species including TG, PC, DG, phosphatidylglycerol, phosphatidylserine, phosphatidylethanolamine, lysophosphatidylethanolamine, lysophosphatidylcholine, and sphingomyelin were identified by a multivariate empirical Bayes approach (MEBA). Triglyceride (15:0/14:0/14:0) had the highest statistical value. Overall, the data indicated that BCS is associated with unique lipid profiles in dairy cow SAT during the peripartal period. Further studies are needed to understand the physiological relevance of these differences.

Key Words: body condition score, lipidomics, adipose tissue

208 Influence of cobalt source, folic acid and rumen-protected Methionine supplementation on performance, metabolism, and liver tissue 1-carbon metabolism biomarkers in peripartal Holstein cows.

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We investigated effects of supplementing an experimental source of Folic acid (FOA; Zinpro Corp.), 2 Co sources [Co glucoheptonate (Copro) or Co pectin (CoPectin), Zinpro Corp.] and rumen-protected methionine (RPM) on performance and metabolism of multiparous Holstein cows from −30 relative to parturition through 30 d of lactation. Multiparous Holstein cows blocked according to the expected calving date, cows within each block were balanced for parity, previous lactation milk yield, and BCS before the close-up. Seventy-two cows (17 to 19 per treatment) were individually fed a basal diet [1.37 Mcal/kg of dry matter (DM), 14.5% crude protein] supplemented with Copro, FOA+Copro, FOA+CoPectin, or FOA+CoPectin+RPM. In each FOA group cows received 50 g FOA. Cobalt treatments delivered 1 ppm Co/kg DM. The RPM was fed at 0.09% of DMI to achieve a ratio of 2.8:1 Lys:Met in the MP. Blood samples and liver tissue were collected at −10, 7, 15, and 30 d relative to parturition. The statistical model used in SAS 9.4 included the random effect of block and fixed effect of treatment, time and interactions. Preplanned contrasts were FOA vs. no FOA, CoPectin vs. Copro, and RPM vs. no RPM. No differences were observed for DMI (13.1 ± 0.5 kg/d prepartum, 15.9 ± 0.8 kg/d postpartum) or milk yield (40.1 ± 1.8 k/d). Cows fed FOA had lower ($P = 0.05$) milk fat and total solids %; whereas RPM led to greater ($P = 0.01$) milk protein %. Plasma cholesterol, fatty acids, and bilirubin were lower ($P \leq 0.05$) and retinol and tocopherol greater ($P \leq 0.05$) in cows fed FOA. In contrast, CoPectin led to lower ($P \leq 0.02$) hydroxybutyrate and nitric oxide, and tended to lower ($P = 0.06$) myeloperoxidase activity and increase antioxidant capacity (FRAP). Among 13 genes in the 1-carbon metabolism pathway measured, abundance of the vitamin B_{12}-dependent enzyme methionine synthase (MTR) was the only one affected, being greater in cows fed CoPectin. Despite similar performance, data revealed alterations in plasma and liver tissue biomarkers when feeding different Co sources with or without FOA and rumen-protected methionine.

Key Words: prepartum, postpartum, vitamin B_{12}

209 Rumen-protected methionine supply to cows during heat stress alters liver tissue protein abundance of mechanistic target of rapamycin.

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The objective was to investigate effects of rumen-protected Met (RPM) during a heat stress (HS) challenge on protein abundance of mechanistic target of rapamycin (mTOR), insulin, and antioxidant signaling in liver tissue. Thirty-two multiparous, lactating Holstein cows (184 ± 59 DIM) were randomly assigned to 1 of 2 environmental (Env) treatment groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc., France; 0.105% DM as top dress) or TMR without RPM (CON)] in a crossover design. The study was divided into 2 periods with 2 phases. During phase 1 (9d), all cows were in thermoneutral conditions (TN;THI = 60 ± 3) and fed ad libitum. During phase 2 (9d), group 1 was exposed to HS using electric heat blankets. Group 2 remained in TN but was pair-fed to HS counterparts. After 14d washout and 7d adaptation period, the study was repeated (period 2) and environmental treatments were inverted relative to phase 2, but dietary treatments were the same. Liver biopsies were performed at the end of each period. Tissue was incubated in Dulbecco's modified eagle medium for 2h and protein extracted. Data were analyzed using PROC MIXED in SAS. An Env × diet was detected for CUL3, an inhibitor of nuclear factor erythroid 2 like 2 (NFE2L2), due to upregulation in CON cows during HS vs. TN. Abundance of CUL3 did not differ due to RPM during HS or TN and tended to be lower than CON, suggesting that RPM alleviated HS-induced oxidative stress. An Env × diet was detected for phosphorylated (p) mTOR ($P = 0.001$); compared with CON, RPM led to greater abundance under TN conditions. However, p-mTOR decreased with RPM but increased with CON in HS. These effects suggested a potential shift in protein synthesis with RPM supply during HS. There was also an interaction for p-AKT ($P < 0.001$); HS cows on the CON diet had greater abundance than all other treatments. This response in CON cows was similar to CUL3, suggesting altered insulin signaling. Overall, preliminary evaluation indicates that RPM supplementation may help cows maintain hepatic homeostasis in mTOR, insulin, and antioxidant signaling in liver during a HS challenge.

Key Words: amino acid, lactation

210 Heat stress and rumen-protected methionine alter whole-blood mRNA abundance of transsulfuration and antioxidant pathway genes.

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The objective was to investigate the effects of rumen-protected Met (RPM) during a heat stress (HS) challenge on whole blood mRNA abundance of genes related to immune and antioxidant responses and the transsulfuration pathway. Thirty-two multiparous, lactating Holstein cows (184 ± 59 DIM) were randomly assigned to 1 of 2 environmental (Env) treatment groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc., France; 0.105% DM as top dress) or TMR without RPM (CON)] in a crossover design. The study was divided into 2 periods with 2 phases per period. During phase 1 (9 d), all cows were in thermoneutral conditions (TN; THI = 60 ± 3) and fed ad libitum. During phase 2 (9 d), group 1 (n = 16) was exposed to HS using electric heat blankets. Group 2 (n = 16) remained in TN but was pair-fed to HS counterparts. After a 14-d washout and 7-d adaptation period, the study was repeated (period 2) and environmental treatments were inverted relative to phase 2 in period 1, while the dietary treatments were the same. Blood was collected from the coccygeal vein approximately 4 h postfeeding on d 8 of phase 2. Data were analyzed using PROC MIXED in SAS. An Env × diet interaction was observed for mRNA abundance of cytathionine β synthase (*CBS*; $P = 0.03$); with RPM cows having greater *CBS* during TN compared with HS. The same Env × diet interaction was detected for the mRNA abundance of superoxide dismutase (SOD) 1 ($P = 0.01$)

and cysteine dioxygenase type 1 (*CDO1*; $P = 0.02$). A tendency for an interaction was observed for the expression of toll-like receptor 2 ($P = 0.09$) due to an increase in abundance in CON cows during HS compared with TN. Regardless of diet, mRNA abundance of *CDO1* and cystathionine gamma-lyase was downregulated by HS ($P < 0.05$) and glutathione synthase tended to be downregulated ($P = 0.08$). Overall, preliminary evaluation suggests that supplying RPM may enhance the antioxidant responses in whole blood. Results also highlight the negative effect of HS on whole blood transsulfuration pathway and antioxidant responses.

Key Words: amino acid, immune response

Ruminant Nutrition: Carbohydrates and Lipids

211 Effects of precision feeding Holstein and Jersey heifers a gradual increase of dietary poultry fat on nutrient digestibility. S. M. Hussein*[1], S. Twyman[1], M. Toledo[1], O. Thomas[1], J. Echesabal[1], R. M. Stockler[2], M. J. Aguerre[1], and G. J. Lascano[1], [1]*Clemson University*, *Clemson, SC*, [2]*Auburn University*, *Auburn, AL.*

The term "precision-feeding" refers to a program that provides heifers with the correct amount of nutrients necessary for adequate growth without affecting future performance. Including poultry fat (PF) can accurately meet caloric requirements and reduce dry matter intake (DMI) when diets are isocaloric and isonitrogenous. However, high fat intake may lead to reduction in fiber digestibility and inadequate rumen fermentation. Yet, reduction of DMI as PF is incorporated into diets can provide similar energy to the animal limiting the negative effects associated with fat inclusion. The objective of this study was to evaluate the effects on nutrient digestion of including different levels of PF inclusion in precision feeding dairy heifers. We hypothesized that including PF would further reduce intake without compromising nutrient digestibility in dairy heifers. Treatments included 55% forage diet with 4 levels of PF inclusion starting with a basal concentration of fat in the diet [3% fat (0% PF); 5% fat (2% PF); 7% fat (4% PF); and 9% fat (6% PF)]. Holstein and Jersey heifers were randomly assigned to treatments and administered according to a split-plot, 4 × 4 Latin square design (21-d periods). Data were analyzed using the MIXED procedure of SAS. There were no differences on OM apparent digestibility coefficients (dC) between the Holstein and Jersey-group. However, NDF dC was higher ($P < 0.01$) in Holstein heifers (68.40 vs. 61.34 ± 1.2%), but the Jersey-group had higher ADF (34.15 vs. 44.58 ± 1.6%), and N dC (90.01 vs. 92.04 ± 0.3%). The inclusion of PF did not affect OM, NDF, and ADF dC. However, the EE and N dC increased linearly with the increased level of PF inclusion. The Holstein-group spent more time to finish their meal ($P < 0.05$) than the Jersey-group (65.33 vs. 59.94 ± 1.6 min), and the PF inclusion showed a linear decrease in the time spent to finish the meal. These results suggest that Holstein utilized NDF more efficiently. In the current study, increasing dietary PF inclusion up to 9% DM reduced DMI without affecting OM and fiber digestibility but increased EE and N dC.

Key Words: precision feeding, poultry fat, heifer

212 In vitro gas production detected differences among corn hybrids at silage maturities. N. Schlau*[1], D. R. Mertens[2], and D. Taysom[1], [1]*Dairyland Laboratories Inc*, *Arcadia, WI*, [2]*Mertens Innovation and Research LLC*, *Belleville, WI.*

In vitro starch disappearance (after 7–9 h) is used to detect differences among corn hybrids in mature grains; however, it is not effective for corn silages (CS). This may be due to differences in the zein matrix in immature vs. mature corn combined with drying and grinding samples for analysis. The objective was to determine whether differences can be detected among corn hybrids at silage maturities if the kernels are quartered to eliminate the effects of drying and grinding. Ears of Mycogen hybrids (7-F2F499 (B), 6-TMF2Q419 (C), and 7-UNI95D58 (FL), with DM ranging from 30.7 to 38.4, 29.2–38.4, and 29.1–37.7%, respectively) were harvested from the 3 identical CS hybrids grown on 6 geographies. Starch contents were 72.0 ± 0.89, 71.5 ± 0.84 and 68.5 ± 1.53%DM for the B, C and FL, respectively. Design was an incomplete randomized block with 4 blocks of 5 samples balanced for DM and starch, which included at least one of each hybrid. Two blocks were measured in triplicate in each of 4 runs. The model $Y = \mu + \text{Run} + \text{Block} + \text{Region} + \text{DM} + \text{Hybrid} + \text{Region} \times \text{DM} + \text{Region} \times \text{Hybrid} + \text{DM} \times \text{Hybrid} + \text{Error}$ was tested using aov() in R. Gas production (GP) was measured for 120h using the Ankom RF system with 60mL Goering and Van Soest (1970) media and 20 mL of blended ruminal fluid from 2 steers fed a 30% starch diet. Lag was shorter for FL vs. C and B (8.27 vs. 9.34 and 9.05 ± 0.22h, respectively, $P < 0.0001$). Fractional rate was faster for FL than C and BMR (5.49, 5.03 and 4.15 ± 0.17%/h, respectively, $P < 0.0001$). The DM × Hybrid was significant for all kinetic variables. For the first 3h, GP was similar for all hybrids. After 6h, GP for FL was higher than C and B (11.6 vs. 9.54 and 9.49 ± 0.75 mL gas/g DM, respectively, $P < 0.0001$) and this difference continued through 36h ($P < 0.05$). After 96h, B produced more gas than FL and C (315 vs. 299 and 295 ± 4.43mL gas/g DM, respectively, $P < 0.0001$) and this difference continued through 120h ($P < 0.0001$). Results indicate that GP of quartered undried kernels can effectively quantify differences among corn hybrids at silage maturities, and that starch in floury hybrids (FL) is more accessible to microbes in early fermentation.

Key Words: digestibility, fermentation, starch

213 Effect of altering the ratio of dietary C16:0 and *cis*-9 C18:1 on production and energetic responses of lactating dairy cows: a meta-analysis. J. M. dos Santos Neto*[1], J. de Souza[2], A. M. Burch[1], and A. L. Lock[1], [1]*Michigan State University*, *East Lansing, MI*, [2]*Perdue AgriBusiness*, *Salisbury, MD.*

We performed a meta-analysis to evaluate the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 on production and energetic responses of lactating dairy cows. Treatments were: 1) CON (control; non-FA supplemented diet); 2) 80:10 (FA supplement blend with 80% C16:0 + 10% *cis*-9 C18:1 fed at 1.5% diet DM); and 3) 60:30 (FA supplement blend with 60% C16:0 + 30% *cis*-9 C18:1 fed at 1.5% diet DM). A meta-regression evaluated the relationships of insulin with energy output to milk and to BW. The data set was assembled from individual observations of 316 Holstein cows from 5 studies at Michigan State University with 80:10 (n = 113) and 60:30 (n = 113) compared with CON (n = 87). The meta-analysis was performed using the PROC MIXED of SAS, including study as random effect. Compared with CON, 80:10 did not affect DMI ($P = 0.26$), plasma insulin concentration ($P = 0.14$), BW gain ($P = 0.23$), or energy output to BW ($P = 0.79$), and increased milk yield (2.8 kg/d, $P = 0.02$), milk fat yield (0.13 kg/d, $P = 0.01$), ECM (3.16 kg/d, $P < 0.01$), milk fat content (0.26%, $P < 0.01$), and milk energy output (2.02 Mcal/d, $P = 0.01$). Compared with CON, 60:30 did not affect DMI ($P = 0.79$), tended to increase BW gain (0.21 kg/d, $P = 0.08$), increased energy output to BW (1.53 Mcal/kg, $P = 0.05$), plasma insulin concentration (0.06 µg/L, $P = 0.01$), milk yield (2.27 kg/d, $P = 0.05$), milk fat yield (0.12 kg/d, $P = 0.01$), ECM (3.03 kg/d, $P < 0.01$), and milk fat content (0.26%, $P < 0.01$). Plasma insulin concentration had a negative linear effect on energy output to milk ($38.6 \pm 1.61 - 7.63 \pm 1.74x$, $P < 0.01$; RMSE = 1.48), and a positive quadratic effect on energy output to BW ($-10.0 \pm 3.63 + 28.6 \pm 7.86x - 15.2 \pm 4.71x^2$, $P < 0.01$; RMSE = 1.85). In conclusion, feeding FA blends between 80:10 and 60:30 increased the yields of milk, milk fat, and ECM compared with CON. Increasing dietary *cis*-9 C18:1 increased plasma insulin concentration, with plasma insulin concentration having a positive relationship with energy output to BW. Altering the dietary ratio of C16:0 and *cis*-9 C18:1 may be a useful strategy to change nutrient partitioning in dairy cows.

Key Words: insulin, oleic acid, palmitic acid

214 The effects of parity and stage of lactation on odd- and branched-fatty acid profile in milk fat from dairy cows. L. L. Sun[1], L. Lei[2], J. T. Brenna[3], Z. H. Wu[1], L. Ma[1], J. C. Xu[4], and D. P. Bu*[1,5], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Hunan Agricultural University*, *Hunan, China*, [3]*Dell Pediatric Research Institute and Departments of Nutrition and of Chemistry, University of Texas at Austin*, *Austin, TX*, [4]*Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences*, *Kunming, China*, [5]*Hunan*

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Dairy products are the main source of odd-and branched-fatty acids (OBCFA) in the Western diet, a group of nutrients with emerging health benefits. Animal diet is known to have important influence on milk fat OBCFA; however, effects of physiological factors have received little attention. The objectives of our study were to examine the effects of parity and stage of lactation on OBCFA profiles in milk fat from Holstein dairy cows, and further to investigate the relationship between OBCFA profiles and yield of milk and milk fat. Holstein dairy cows (n = 184) with an average parity of 2.8 ± 0.3 and 173 ± 24 d in milk (DIM) were selected. All cows were fed the same diet, and milk samples were collected on the same day to avoid the effects of diet and season factors. Fatty acid methyl esters contents were analyzed by gas chromatography. A regression model was developed by linear model procedure (PROC GLM) of SAS to analyze the data, and the relationships between OBCFA profiles and yield of milk and milk fat were analyzed by correlation procedure (PROC CORR). Frequency distributions demonstrated that milk fat content of *iso*-15:0, *iso*-17:0, *anteiso*-15:0 and *anteiso*-17:0 varied 3-4-fold among individuals. Parity and DIM accounted for less than 25% of total individual variation of OBCFAs, except for n-15:0 (26.8%). Parity and DIM had significant effects on some OBCFAs, including n-15:0 ($P < 0.001$), *iso*-13:0 ($P < 0.01$), *iso*-15:0 ($P < 0.01$), *iso*-17:0 ($P < 0.001$), *anteiso*-15:0 ($P < 0.01$), and *anteiso*-17:0 ($P < 0.01$). Negative correlations existed between n-13:0 ($r = -0.22$, $P = 0.03$), *iso*-14:0 ($r = -0.21$, $P = 0.03$), and *iso*-16:0 ($r = -0.22$, $P = 0.03$) and yield of milk and milk fat. There were no relationships between milk yield or milk fat and other OBCFAs of Holstein dairy cows.

Key Words: dairy cow, odd- and branched-chain fatty acid, parity

215 Profiles of odd- and branched-chain fatty acids in bovine colostrum and transition milk. H. S. Xin[1,2], Y. Xu[2], Y. H. Chen[2], G. Chen[2], and L. L. Guan*[2], [1]*College of Animal Science and Technology, Northeast Agricultural University, Harbin, Heilongjiang, China*, [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.*

Odd- and branched-chain fatty acids (OBCFAs) including iso-C14:0, iso-C15:0, C15:0, anteiso-C15:0, iso-C16:0, iso-C17:0, C17:0, anteiso-C17:0 and iso-C18:0 are found in bovine milk and have been reported to have positive influences on human health. However, it is unknown whether they are also present in colostrum and transition milk, which are used to feed neonatal calves and are potential bioactive compounds impacting calf gut health. Therefore, this study aimed to investigate OBCFA profiles in colostrum and transition milk. All samples were collected from a total of 12 Holstein dairy cows (6 primiparous and 6 multiparous) on the 1st day (colostrum, within 0.5 h), 3rd day (transition milk) and 5th day (milk) after calving, respectively. The OBCFAs profiles of the milk samples were measured with gas chromatography and the effect of milk type on OBCFAs was analyzed using the PROC MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC). The total OBCFAs amount was 134 mg/100g milk in the colostrum, which was 24% and 35% lower ($P < 0.05$) than that in the transition milk and milk, respectively. Similar findings were found on the contents of total iso-OBCFAs, total anteiso-OBCFAs, total odd-OBCFAs, total even-OBCFAs as well as total BCFAs. As for the individual OBCFA profiles, straight-chain fatty acids C15:0 and C17:0 were the top 2 abundant OBCFA in all milk types, accounting for 20–25% and 21–24% of the total OBCFAs, respectively. The proportions of iso-C14:0, anteiso-C15:0, iso-C16:0, iso-C17:0, anteiso-C17:0 and iso-C17:0 were the lowest ($P < 0.05$) in the colostrum, followed by the transition milk and milk, while the proportions of iso-C15:0 and C15:0 were the highest in the colostrum, followed by the transition milk and milk. No difference was observed on iso-C18:0 proportion among 3 milk types. In summary, the results indicated that colostrum, transition milk and milk had different OBCFAs contents and profiles with the total OBCFAs content being lower in colostrum, compared with transition milk and milk samples. Whether these differences play a role in calf health warrants future studies.

Key Words: odd- and branched-chain fatty acids, colostrum, transition milk

216 Effect of a low forage diet on the yields of milk and milk components and feed efficiency of mid-lactation dairy cows. A. N. Negreiro* and A. L. Lock, *Michigan State University*, *East Lansing, MI.*

Examining the effect of lower forage diets and alternative fiber sources in lactating dairy cow diets is important since forage quality and quantity can vary greatly depending on growing conditions, years, and locations. Therefore, we determined the effect of feeding diets similar in NDF, starch, and CP with differing amounts of forage on the yields of milk and milk components of mid-lactation dairy cows. Thirty-two Holstein cows (132 ± 68 DIM) were used in a crossover design with 2 consecutive 28-d periods, with sample and data collection during the final 5 d of each period. Treatment diets were: 1) control diet (CON) containing high forage (51.4% diet DM; forage NDF 18% diet DM) and no supplemental fat or supplemental amino acids (RUP 6.67% diet DM; RDP 10.4% diet DM); and 2) low forage diet (LF) containing low forage (31.6% diet DM; forage NDF 11.2% diet DM), including supplemental fat (1.5% diet DM; 82% C16:0-enriched supplement) and supplemental amino acids (RUP 7.01% diet DM; RDP 9.69% diet DM). Diets were balanced for similar NDF (29% diet DM), starch (26.5% diet DM), and CP (16.9% diet DM). The statistical model included the random effect of cow and fixed effects of diet, period, and their interaction. Results are presented in the sequence CON vs LF. There was no effect of treatment on milk yield (45.4 vs 46.1 kg/d, $P = 0.34$), milk fat content (3.95% vs 3.99%, $P = 0.38$), or BW (704 vs 703 kg, $P = 0.83$). Compared with CON, LF decreased DMI (34.7 vs 28.6 kg/d, $P < 0.01$) and increased milk fat yield (1.78 vs 1.84 kg/d, $P = 0.02$), milk protein yield (1.47 vs 1.56 kg/d, $P < 0.01$), milk protein content (3.24% vs 3.41%, $P < 0.01$), ECM (48.3 vs 50.2 kg/d, $P < 0.01$), feed efficiency (ECM/DMI; 1.41 vs 1.77 kg/d, $P < 0.01$), and BCS (3.2 vs 3.3, $P = 0.02$). Our results demonstrate that feeding a low forage diet supplemented with amino acids and a palmitic acid-enriched supplement increased feed efficiency through decreased DMI and increases in the yields of milk fat and protein, without changes in BW.

Key Words: forage, amino acid, fatty acid

217 Predicting the yield of milk fat and milk fatty acid sources from fatty acid intakes in lactating dairy cows: A meta-analysis. J. M. dos Santos Neto*[1], J. de Souza[2], and A. L. Lock[1], [1]*Michigan State University*, *East Lansing, MI*, [2]*Perdue AgriBusiness*, *Salisbury, MD.*

We performed a meta-analysis to evaluate the effect of intake (_i) of C16:0, C18:0, C18:1, C18:2, and C18:3 on the yields of milk fat and milk fatty acid (FA) sources. Our analysis used 1,339 individual cow observations from 16 studies at Michigan State University. Diets (% DM) contained (mean ± SD) 30.1 ± 2.47 NDF, 26.6 ± 3.17 starch, and 4.03 ± 0.78 total FA. Total FA_i averaged 1,126 g/d ± 296 and ranged from 308 to 2,248 g/d. Statistical analyses were performed using the PROC MIXED of SAS, including random effects of study, period within study, and cow within study. Individual FA_i (g/d) were used as independent variables, the yields (g/d) of milk fat and milk FA were used as dependent variables. Independent variables were removed from the model using backward elimination with significance criteria of $P > 0.10$. Total FA intake was not used as an independent variable in the model to avoid multicollinearity. We classified milk FA by source as de novo (<16 carbons), mixed (16 carbons), and preformed milk FA (>16 carbons). C16:0_i linearly increased the yield of milk fat [1,552 ± 70.9 + 0.30 ± 0.04 x C16:0_i, $P < 0.01$; RMSE = 1.30]; C18:3_i tended to linearly decrease the yield of de novo milk FA [373 ± 18.8 - 0.04 ± 0.03 x C18:3_i, $P = 0.10$; RMSE = 19.3], C16:0_i linearly increased mixed milk FA [468 ± 19.7 + 0.22 ± 0.01 x C16_i, $P < 0.01$; RMSE = 14.5], and C18:1+C18:2_i tended to linearly increase preformed milk FA [486 ± 48.9 + 0.33 ± 0.12 x C18:1_i + 0.13 ± 012 x C18:2_i, $P = 0.06$; RMSE = 22.4]. C18:0_i had no effect on milk FA yield ($P > 0.10$). Among individual de novo milk FA, C16:0 tended to linearly decrease milk FA from 10 to 14-carbons ($P = 0.07$), had no effect on C8:0 ($P = 13$), and linearly increased the yields of C4:0 [43.6 ± 2.94 + 0.009 ± 0.001 x C16:0_i, $P < 0.01$; RMSE = 1.61] and C6:0 [29.5 ± 1.55 + 0.002 ± 0.001 x C16:0_i, $P = 0.02$; RMSE = 0.96]. In conclusion, the yields of milk FA by source were dependent on the profile of FA ingested. Overall, C16:0_i was associated with increasing milk fat yield. C16:0 increased C4:0 and C6:0, which can be associated with the role of these FA in maintaining milk fluidity, given their low melting point.

Key Words: fatty acid, meta-analysis, milk fat

Ruminant Nutrition: Gut Physiology, Fermentation, and Digestion

218 Effects of rumen-protected choline on hepatic metabolism during induction of fatty liver. U. Arshad*, M. B. Poindexter, A. Husnain, R. Zimpel, M. C. Perdomo, A. Vieira-Neto, and J. E. P. Santos, *University of Florida*, *Gainesville, FL.*

Objectives were to determine the effects of rumen-protected choline (RPC) on hepatic composition and plasma metabolites in cows induced to develop fatty liver. The hypothesis was that RPC would reduce triacylglycerol (TAG) and increase glycogen contents in liver. Pregnant, nonlactating parous Holstein cows (n = 110) were blocked by BCS (4.02 ± 0.5) at 232 d of gestation and assigned randomly (22/treatment) to receive choline chloride (CC) supplemented as RPC with either a low (L, 28.8% CC) or high concentration (H, 60.0% CC) in the RPC product. The amounts supplemented were 0 (CON), 12.9, or 25.8 g/d of choline ion resulting in 5 treatments, CON, L12.9, L25.8, H12.9, and H25.8 fed for 14 d. Cows were fed for ad libitum intake on d 1 to 5, and restricted to 50% of the NE_L required from d 6 to 14. Intake of metabolizable methionine was maintained at 20 g/d for the 14 d. Liver was sampled on d 6 and 13 and analyzed for TAG and glycogen. Blood was sampled throughout the experiment. Data were analyzed by ANOVA with mixed models using the MIXED procedure of SAS. Contrasts included the effects of choline (CON vs. L12.9+L25.8+H12.9+H25.8), source (L12.9+L25.8 vs. H12.9+H25.8), amount (L12.9+H12.9 vs. L25.8+H25.8), or the interaction (L12.9+H25.8 vs. L25.8+H12.9). Supplementing RPC reduced hepatic TAG and increased glycogen contents without affecting plasma concentrations of NEFA or BHB (Table 1). Increasing the dose of choline ion from 12.9 to 25.8 g/d further reduced TAG and increased glycogen contents. Choline improved hepatic composition of cows in negative nutrient balance independent of changes in plasma metabolites.

Table 1 (Abstr. 218). Responses to treatments during feed restriction

Item	CON	L12.9	L25.8	H12.9	H25.8	SE
NE intake, Mcal/d	7.05	7.04	6.81	7.29	7.09	0.20
Liver TAG, %						
As is*†	9.32	6.59	5.05	6.61	6.00	0.55
DM*†	26.33	18.59	14.55	18.29	16.75	1.65
Liver glycogen, %						
As is*‡†	1.83	2.59	3.55	3.13	4.07	0.18
DM*‡†	5.01	7.26	10.32	8.63	11.58	0.57
TAG:glycogen						
As is*†	5.19	2.39	1.29	2.02	1.35	0.31
DM*†	5.20	2.40	1.29	2.03	1.36	0.31
Plasma						
NEFA, m*M*	0.837	0.854	0.835	0.848	0.777	0.062
BHB, m*M*	0.801	0.779	0.758	0.769	0.760	0.061

Effect $P < 0.05$: *choline; ‡concentration; †amount; §interaction.

Key Words: choline, fatty liver, triacylglycerol

219 Effects of different weaning strategies when feeding moderate and high milk replacer rates on fecal bacteria taxonomic profile, diversity, and community structure in Holstein calves. A. Poulin*[2], J. Romero[1], R. Klopp[3], V. Richards[4], F. Suarez-Mena[5], T. Dennis[5], T. Hill[5], R. Causey[1], R. Schlotterbeck[5], and G. Lascano[3], [1]*Animal and Veterinary Sciences, SFA, University of Maine*, *Orono, ME*, [2]*Department of Molecular and Biomedical Sciences, University of Maine*, *Orono, ME*, [3]*Department of Animal and Veterinary Sciences, Clemson University*, *Clemson, SC*, [4]*Department of Biological Sciences, Clemson University*, *Clemson, SC*, [5]*Nurture Research Center, Provimi*, *Brookville, OH.*

Fecal bacteria profile can be greatly affected by pre-weaning diet and the strategy (STEP) used to wean calves. A 2 × 2 factorial design [moderate (MOD) or high (HI) milk replacer (MR) feeding rates and one step (ONE) or gradual (GR) weaning] was utilized to study how pre-weaning programs affect fecal bacteria diversity from 2 to 4 mo of age. Calves (n = 50, 56 d of age) were randomly assigned to 1 of the 4 pre-weaning treatments. The initial BW for HI and MOD calves was 78.9 and 74.3 ± 1.92 (kg), respectively. Bacterial diversity was determined using the 16S rRNA gene (V4 region) for 0, 14, 28, 42, 56 d after weaning. The statistical model included the fixed effects of MR, STEP, DAY and their interactions and the random effect of calf nested within treatment. Differences were declared at $P \leq 0.05$. At HI feeding, *Ruminococcaceae* relative abundance (rA, %) was reduced with ONE vs. GR weaning (15.8 vs. 18.5 ± 1.0%); however, no differences were observed in MOD feeding (~16.9). For ONE weaning, HI feeding reduced *Lachnospiraceae* compared with MOD (6.8 vs. 9.4 ± 0.83%) but no differences occurred within GR (~7.9). At d 0, ONE weaning increased *Bacteroidaceae* relative to GR (24.6 vs. 10.0 ± 6.3%). As calves went from 0 to 56 d postweaning, the rA of *Ruminococcaceae* was lower for d 0 vs. 14 to 56 (11.9 vs. ~18.4 ± 1.2%); unidentified *Bacteroidales* was lower at d 42 vs. 0 to 28 (5.6 vs. ~12.4 ± 1.7); *Lachnospiraceae* was lower for d 0 vs. 14 to 56 (4.6 vs ~9.1 ± 0.94); and *Prevotellaceae* was lower for d 28 vs. 0,14,42, and 56 d (4.0 vs ~8.0 ± 1.2%). At HI feeding, ONE reduced phylogenetic diversity (richness) relative to GR (20 vs. 21.3 ± 0.55%). A PERMANOVA analysis of weighted UNIFRAC distances indicated a difference in community structure across treatment and period combinations ($P < 0.01$). In conclusion, both MR feeding rate and weaning steps influenced bacterial rA independent of time, except for *Bacteroidaceae*; nonetheless both these factors had no effect on fecal bacteria richness.

Key Words: calves, post-weaning, fecal microbiome

220 Effects of acute intravenous trimethylamine *N*-oxide infusion on plasma and serum markers of liver health, glucose tolerance, and milk production in early lactation cows. W. A. Myers*[1], F. Wang[1,2], C. Chang[1], A. N. Davis[1], J. E. Rico[1], B. N. Tate[1], L. F. Wang[1,3], and J. W. McFadden[1], [1]*Cornell University*, *Ithaca, NY*, [2]*China Agricultural University*, *Beijing, China*, [3]*Henan Agricultural University*, *Zhengzhou, China*.

Choline and carnitine are degraded in the gastrointestinal tract in part to trimethylamine, which is oxidized in the liver to form trimethylamine *N*-oxide (TMAO). In non-ruminants, TMAO has been implicated in the development and progression of fatty liver, heart and kidney disease, diabetes, and cancer. However, the effects of TMAO in cows required investigation. Therefore, 8 early lactation Holstein cows (30.4 ± 6.41 d in milk; 2.88 ± 0.83 parity) were enrolled in a study with a 4 × 4 replicated Latin square design. Cows were intravenously infused TMAO at 0 (control), 20, 40, or 60 g/d for 6 d. Washout periods lasted 9 d. An intravenous glucose tolerance test (GTT) occurred on d 5. Blood was collected daily. Milk was collected on d −1, 0, 5, and 6. Urine was collected on d −1 and 6. Plasma TMAO, triglyceride, fatty acid, and glucose concentrations were quantified. A serum liver panel analysis and milk composition were also analyzed. The mixed model included the fixed effects of baseline measurements as a covariate and treatment. Dry matter intake was not modified by treatment. Plasma, milk, and urine TMAO concentrations increased linearly with increasing dose (e.g., 12 to 204 μM in cows infused 0 and 60 g of TMAO/d, respectively; $P < 0.01$). Majority of TMAO was excreted in urine. Plasma triglyceride, fatty acid, and glucose concentrations were not modified by treatment. Serum albumin, total protein, globulin, total bilirubin, direct bilirubin, aspartate aminotransferase, gamma-glutamyl transferase, and glutamate dehydrogenase concentrations were not modified by treatment. Serum glutamate dehydrogenase concentrations decreased linearly with increasing dose ($P < 0.05$). Serum GTT glucose and fatty acid concentrations were not modified by treatment. Milk yield and composition (fat, protein, and lactose), energy-corrected milk, and feed efficiency were not modified by treatment. We conclude that the acute intravenous infusion of TMAO does not modify measures of liver health, glucose tolerance, or milk production in early lactation cows.

Key Words: choline, dairy cow, trimethylamine *N*-oxide

221 Effects of milk replacer plane of nutrition and levels of starch and neutral detergent fiber in pelleted starter on whole gastrointestinal tract pH around weaning. T. T. Yohe*[1], T. S. Dennis[2], J. D. Quigley[2], T. M. Hill[2], F. X. Suarez-Mena[2], K. M. Aragona[2], J. H. Costa[3], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph*, *Guelph, ON, Canada*, [2]*Nurture Research Center, Provimi, Cargill Animal Nutrition*, *Brookville, OH*, [3]*Dairy Science Program, Animal and Food Sciences, University of Kentucky*, *Lexington, KY.*

The objectives of this study were to examine the effects of pelleted starter diets differing in starch and NDF content when fed differing levels of milk replacer (MR) on gastrointestinal pH rumen pH around weaning in dairy calves. The 4 dietary treatments (Trt; n = 12 per trt) were: 691 g MR/day (dry matter basis; DM) with starter containing low or high starch (LL:12.0% and LH:35.6% starch, respectively) and 1,382 g MR/day (DM) with starter containing low or high starch (HL and HH, respectively). All calves were in individual pens with straw bedding until wk 5 when bedding was covered. Calves were fed MR twice daily (0700 and 1700 h) containing 24.5% CP (DM), 19.8% fat (DM), and had ad libitum access to starter and water. Calves arrived between 1 and 3 d of age and the study lasted 8 wk with calves undergoing step-down weaning during wk 7. Starting at wk 5, an indwelling pH logger was inserted orally to monitor rumen pH until calves were dissected at the end of the study in wk 8. Data were analyzed using PROC MIXED in SAS 9.4 with the fixed effects of Trt, week, and their interaction, and random effect of calf nested within Trt. There were no differences between Trt or interaction within week for time below rumen pH 5.8, but there were trending differences between Trt for time below rumen pH 5.0 and 4.5 (Table 1). There were also Trt differences in rumen and abomasal pH at dissection (Table 1). Modest differences were present between Trt, suggesting further research and refinement of diets (altering feed particle size) may help our understanding of the effects of plane of MR and starch levels on gut development and health in calves.

Key Words: dairy, calf nutrition, fermentation

Table 1 (Abstr. 221). Gastrointestinal tract pH around weaning

	Trt					*P*-value		
Item	LL	LH	HL	HH	SEM	Trt	Wk	Trt × Wk
Time below rumen pH, min/d								
5.8	771.62	718.37	539.34	684.25	82.77	0.21	<0.01	0.03
5.0	39.04	240.30	26.89	64.86	64.85	0.08	0.05	0.38
4.5	1.27	124.89	0.21	6.20	42.28	0.10	0.19	0.37
pH at dissection								
Rumen	5.50^{ab}	5.14^{b}	5.63^{a}	5.60^{a}	0.12	0.02	—	—
Abomasum	2.87^{b}	3.66^{a}	3.22^{ab}	3.48^{ab}	0.20	0.04	—	—

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

222 Unprotected choline chloride alters microbial community composition in a dual-flow continuous culture system. J. A. Arce-Cordero*[1], P. Fan[1], H. F. Monteiro[1], X. Dai[1], B. Calvo[2], R. Lobo[1], K. Estes[3], K. C. Jeong[1], and A. P. Faciola[1], [1]*Department of Animal Sciences, University of Florida*, *Gainesville, FL*, [2]*Department of Animal Sciences, Maringa State University*, *Maringa, Parana, Brazil*, [3]*Balchem Corporation*, *New Hampton, NY.*

Choline (Cho) is usually fed as rumen-protected Cho to prevent Cho ruminal microbial degradation and assure Cho supply for absorption in the small intestine; however, some Cho is degraded in the rumen and its effects on ruminal microbial population are unknown. Therefore, our objective was to evaluate the effects of unprotected choline chloride on ruminal microbial community composition when fed to diets with different NDF concentration. We used 8 fermenters of a dual-flow continuous culture system in a 4x4 duplicated Latin-square with a 2x2 factorial arrangement; factors being: choline chloride supplementation (0 and 2.5 g/kg of DM) and dietary NDF% (30% and 40% of DM). Resulting treatments were: 1) 30% NDF + choline; 2) 30% NDF, without choline; 3) 40% NDF + choline, 4) 40% NDF, without choline. Basal diets were fed at 0800 and 2000, and choline chloride was provided 4 times/d. Each experimental period lasted 10 d. Bacterial samples were collected on d 8, 9, and 10 at 3, 6, and 9 h after morning feeding and a composite sample per time point was made for each fermenter. Particle associated bacteria (PAB) and liquid associated bacteria (LAB) were analyzed separately. Sequencing of V4 region of bacterial 16s rRNA was performed with Illumina MiSeq and data were analyzed with QIIME, R, and SAS. Main effects of choline supplementation (Cho), dietary NDF% (NDF), and their interaction (Cho × NDF) were tested. Cho supplementation impacted relative abundance (RA) of taxonomic orders by increasing *Selenomonadales* RA in PAB ($P = 0.04$) and decreasing *Fibrobacterales* RA in LAB ($P = 0.05$). At the genus level, Cho increased RA of *Ruminococcaceae* ($P = 0.02$), *Megasphaera* ($P = 0.05$) and *Prevotella* ($P = 0.08$) in PAB; while *Succinivibrio* RA in PAB was greater in response to Cho only when the 30% NDF diet was provided (Cho × NDF, $P = 0.02$). Our results indicate that unprotected choline chloride alters ruminal microbial populations associated with carbohydrate fermentation by increasing RA of bacteria associated with starch utilization and propionate synthesis.

Key Words: 16s rRNA, in vitro, methyl donor

Animal Behavior and Well-Being

223 Calf welfare views and dairy consumption habits of parents compared with their children. R. Perttu*, B. Ventura, and M. Endres, *Department of Animal Science, University of Minnesota*, *St. Paul, MN*.

The objective of this study was to explore views of dairy calf welfare and dairy product consumption habits among American parent/child pairs. Parents and their children ($n = 188$ pairs) independently completed a mixed-methods survey administered in-person at the Minnesota State Fair. The survey included a series of multiple-choice questions related to demographics, an open-ended question on 'what dairy calves need to have a good life', and multiple-choice questions about participants' consumption of dairy products and plant-based beverages. Content analysis was used for responses to the open-ended question, and themes were created to describe calf welfare values. The relationship between parent and child responses to the question on 'what dairy calves need to have a good life' and the questions on consumption habits were investigated using Cohen's Kappa analysis. Median age range of the parent participants was 34–44 yr, 70% were female, 79% urban residents, 82% did not have prior experience with agricultural animals, and 71% had visited a farm. For children, the median age of participants was 10 yr, 63% were female, 79% were urban residents, 60% did not have prior experience with agricultural animals, and 82% had visited a farm. In response to 'what dairy calves need to have a good life', parent participants mentioned themes related to biological functioning (80% of respondents), followed by natural living (62%), humane care (26%), and affective state (3%). Children participants mentioned elements related to biological functioning (91%), followed by natural living (55%), humane care (34%), and affective state (5%). The parent/child relationship was associated with natural living (Kappa = 0.21; $P = 0.004$; overall agreement = 61%). In addition, the parent/child relationship was a factor for predicting the consumption of plant-based beverages (Kappa = 0.14; $P = 0.05$; overall agreement = 57%). It appears that parents and children are influencing each other mostly on views related to natural living of calves. However, we suggest that elements related to biological functioning, such as feed, water, shelter, were considered essential to the welfare of dairy calves by the majority of participants.

224 Disbudding and dehorning practices for pre-weaned dairy calves by farmers in Wisconsin. J. Saraceni*[1], J. Van Os[2], C. Miltenburg[3], E. Nelson[4], D. Renaud[1], C. Winder[1], M. Akins[2], T. Ollivett[5], T. Kohlman[6], H. Schlesser[6], B. Schley[6], S. Stuttgen[6], and J. Versweyveld[6], [1]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*, [2]*Department of Dairy Science, University of Wisconsin-Madison*, *Madison, WI*, [3]*Ontario Ministry of Agriculture, Food and Rural Affairs*, *Guelph, ON, Canada*, [4]*Department of Sociology and Anthropology, University of Guelph*, *Guelph, ON, Canada*, [5]*School of Veterinary Medicine, University of Wisconsin-Madison*, *Madison, WI*, [6]*Division of Extension, University of Wisconsin-Madison*, *Madison, WI*.

The objective of this study was to understand common dehorning and disbudding practices in Wisconsin to better target extension education programming to encourage adoption of best practices. A survey was distributed in 2019 to dairy farmers and calf raisers, both online and at extension events. Of all Wisconsin respondents ($n = 188$), producers reported milking a mean of 486 cows and had a mean of 110 heifer calves being fed milk. Respondents could select more than one method of disbudding or dehorning if used on their calves. A total of 126 producers (67%) reported using caustic paste for disbudding, with application most commonly occurring on the day of birth (64; 51%) or between 1 d to 1 wk of age (52; 41%). Hot iron disbudding was reported by 112 producers (60%) and disbudding occurred most commonly at 8 weeks of age or older (39; 35%) followed by between 4 to 8 weeks of age (38; 34%), and between 1 to 4 weeks of age (30; 27%). Of 40 producers reporting surgical amputation methods, 37 (93%) reported disbudding at 8 weeks of age or older. Response rate for the question regarding pain control was 99% (187/188). A total of 85 (45%) respondents reported using pain control, with 28 (33%) using a combination of medications. When each medication was evaluated individually, 40 respondents reported using a local anesthetic (21%), 65 reported using an NSAID (35%) and 10 reported using a sedative (5%). Response rate for questions regarding changes to pain control was 90% (170/188). Over half of the respondents (96/170) reported their use of pain control medications had changed in the last 10 years, with the most common cited influence of change being their veterinarian (66; 68%), and public perception/consumer demand (28; 29%). Results of this study demonstrate a proportion of surveyed farmers continue to perform disbudding without pain mitigation. These findings suggest a need for targeted extension education programming to encourage the implementation of best practices for pain-control and to motivate farmers to perform disbudding at an earlier age.

Key Words: survey, welfare, anesthetic

225 Use of a shelter in group-housed calves shows consistency over time and is affected by disbudding. K. N. Gingerich*, E. E. Lindner, L. M. Coll-Roman, and E. K. Miller-Cushon, *University of Florida*, *Gainesville, FL*.

Changes in social behavior may provide insight into individual experiences of pain. We provided group-housed calves two 3-sided, open top shelters (1.2 × 1.2 m), constructed from corrugated plastic, and examined how shelter use was affected by disbudding. Holstein bull and heifer calves ($n = 16$) were housed in groups of 8 (4 focal calves/pen in addition to 4 non-study calves; 4 pens total). Calves were randomly assigned within pen to be disbudded (DB; $n = 8$) or receive handling only (CON; $n = 8$) at 41 ± 3 d. Calves received local anesthetic and analgesic before disbudding. Behavior was recorded continuously from video for 24 h at 30 ± 5 d of age, during the week before treatment, and for 72 h after treatment to characterize the number of visits to the shelter (>50% of body inside shelter) and visit duration. Data were summarized by day and analyzed in a general linear mixed model with fixed effects of treatment (DB or CON), day as a repeated measure, and pen as a random effect. Shelter use on the observation day preceding treatment was included as a covariate, and we examined correlation between shelter use in both weeks by treatment using linear regression. All calves entered a shelter at least once during the observation period but there was considerable individual variability in use duration (min = 1.4 min/d; max = 13.6 h/d) and visit frequency (min = 1, max = 25 visits/d). Disbudding did not affect frequency of shelter visits (8.8 visits/d; SE = 1.5; $P = 0.56$), but visits tended to be longer for DB calves (32.5 vs. 19.2 min; SE = 8.9; $P = 0.07$). Behavior during the week before treatment was a significant predictor of shelter visit frequency and duration ($P < 0.05$). Specific regression outcomes suggested that shelter use duration and visit characteristics were consistent between weeks for CON calves ($P < 0.002$) whereas shelter visit characteristics for DB calves were not correlated between weeks, further suggesting that disbudding altered shelter use. These results suggest that use of a shelter is affected by disbudding, possibly reflecting a changing motivation for social contact, and that facilitating broader behavioral expression may improve welfare.

Key Words: dairy calf, disbudding, social behavior

226 The effects of xylazine sedation in 2- to 6-wk-old calves disbudded with a cautery iron. C. N. Reedman*[1], T. F. Duffield[1], T. J. DeVries[2], K. D. Lissemore[1], and C. B. Winder[1], [1]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*, [2]*Department of Animal Biosciences, University of Guelph*, *Guelph, ON, Canada*.

The use of local anesthesia and nonsteroidal anti-inflammatory drug (NSAID) analgesia can reduce indicators of pain and inflammation and support self-rewarding behaviors in calves following disbudding. Although the use of sedation may be recommended as best practice for disbudding, there is little research in this area. The objective of this study was to evaluate the effects of xylazine sedation given with a local anesthetic and NSAID in calves following cautery disbudding. Outcomes included automated feeder parameters, pressure sensitivity (measured by a pressure force algometer) and struggle behavior (by blinded observer). One hundred and 22 female and male Holstein calves aged 13 to 44 d were enrolled over 9 blocks and randomly allocated to 1 of 2 treatments: 1) sedated: lidocaine cornual nerve block, meloxicam and xylazine, or 2) non-sedated: lidocaine cornual nerve block and meloxicam. Data were analyzed using mixed models with a fixed effect for baseline values and a random effect for trial block. Linear regression was used to assess continuous outcomes and logistic regression to assess binary outcomes. Sedated calves had reduced average drinking speed at 0 to 24 h and 24 to 48 h following disbudding compared with non-sedated calves (24 to 48 h; −40.9 mL/min, 95% CI −76.8 to −4.9, $P = 0.03$) but there was no difference between groups in total amount of milk consumed daily ($P = 0.86$). Sedated calves had reduced pressure sensitivity ($P < 0.01$) at 0, 60- and 240-min post-disbudding (0 min; −0.37 kgf, 95% CI −0.49 to −0.25; 60 min; −0.69 kgf, 95% CI −1.03 to −0.35; 240 min; −0.72 kgf, 95% CI −1.16 to −0.28) but there were no detected differences between groups 24 h after disbudding ($P = 0.42$). During the disbudding procedure, non-sedated calves had 4.5 (95% CI 1.5 to 13.2, $P = 0.006$) times the odds of struggling more than twice compared with sedated calves. The results indicate that xylazine sedation, in conjunction with a local anesthetic and NSAID, can reduce behavioral indicators of pain in calves disbudded with a cautery iron, but also appears to impact suckling behavior for at least 48 h following sedation.

Key Words: dairy calf, disbudding, behavior

227 Effects of two methods of castration on the growth and intake of dairy calves.

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Dairy bull calves kept for beef production are usually castrated to reduce aggression, mounting behaviors, and to improve meat quality. Two common methods of castration are surgery (resulting in complete removal of the testes) and application of a rubber ring (causing constriction of the blood flow, leading to tissue death and eventual slough off). The aim of this study was to assess and compare wound healing, BW and feed intake over 8 weeks following both methods. Holstein bull calves (n = 22) were enrolled and randomly assigned to treatment. All calves were castrated at 28 (±1) days old, and always with multi-modal pain mitigation (including the use of a sedative, local anesthetic and NSAID). Feed intake and BW were recorded for 3 d before and after castration, and once weekly thereafter for 8 wks. Wound condition was evaluated the day after castration, and weekly thereafter for 8 wks. Wounds of surgically castrated calves healed 28 (±6) days after the procedure (i.e., incision no longer visible); in contrast, the necrotic tissues of the rubber ring calves sloughed off 50 (±9) days after the ring was applied, and the wound was not fully healed in the 2 weeks following slough off. Eight weeks after castration, surgically castrated calves had gained 51.1 (±11.5) kg versus 40.2 (±13.6) kg for those with a rubber ring ($P = 0.019$). Average daily milk intake of all calves in each treatment was calculated, and 2 linear models were built: no significant difference was found between treatments ($P = 0.057$). Similarly, quadratic models were built for grain intakes in both treatments, and ANOVA was carried out: there was a significant difference between treatments ($P < 0.001$). Calves assigned to the rubber ring treatment consumed less grain than surgically castrated calves over the 8-wk period. These results indicate that calves recover more rapidly after surgical castration, as evidenced by improved wound healing, BW gain and feed intake in the weeks following the procedure.

Key Words: post-operative pain, banding, growth

228 Effects of pair or single housing on performance of dairy calves in outdoor hutches.

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Social housing of pre-weaned dairy calves indoors has shown benefits for their welfare, growth, and solid feed intake, which is important for rumen development. Recent research has begun to explore pair raising calves using adjacent outdoor hutches with a shared fence. Our objective was to evaluate pair vs. individual hutch housing effects on performance before and during weaning. Eighty Holstein heifers were individual (n = 16 calves) or pair housed (n = 32 pairs). Calves had ad libitum access to starter. During the pre-weaning period (up to 41 ± 1 d of age, mean ± SD), calves were fed 3.8 L of pasteurized milk 2 times/d. Calves were weaned in a step-down fashion and fed 1.9 L of milk 2 times/d for 7 d, 1.9 L 1 time/d for 4 d, and were completely weaned by 52 ± 1 d of age. Body weights, body-frame dimensions (wither height, hip height, body length, and heart girth) and starter intakes (adjusted for DM%) were measured weekly and averaged within each pair for pair-housed calves. For final BW and body-frame dimensions, linear mixed models were used to evaluate treatment effects, with baseline measurements at 0 d old (BW) and 11 ± 2 d old (body-frames) as a covariate. For starter DMI and ADG, treatment and week effects and their interaction were evaluated separately for the pre-weaning and weaning periods (both measures), and 1 week post-weaning (DMI); overall treatment effects were evaluated for the entire period when each measure was collected. Overall, paired calves tended to have higher starter DMI (pair vs. individual: 0.71 ± 0.03 vs. 0.59 ± 0.05 kg/d, $P = 0.07$), driven by differences during the weaning period (pair vs. individual: 1.31 ± 0.07 vs. 1.01 ± 0.10 kg/d, $P = 0.03$). During the pre- and post-weaning periods, DMI did not differ between treatments ($P > 0.31$). ADG did not differ overall or during either milk-feeding period ($P > 0.58$). Final BW and body-frame dimensions did not differ between treatments ($P > 0.97$). Paired and individual calves had similar growth performance, but during weaning paired calves consumed more solid feeds. These results suggest that calves reared in hutches with a social companion transition better to solid feeds during weaning.

Key Words: social housing, growth, weaning

Animal Health: Calves 2

229 Fecal microbiome profiles of pre-weaned Jersey and Holstein calves with gastrointestinal disease. G. S. Slanzon*, L. M. Parrish, S. C. Trombetta, W. M. Sischo, and C. S. McConnel, *Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University*, *Pullman, WA*.

Gastrointestinal (GI) disease is the most common illness in pre-weaned dairy calves. The fecal microbiome composition is associated with health status, but changes in the microbiome across different levels of GI disease and different breeds remain unclear. Our objective was to associate symptoms of GI disease and breed differences with the fecal microbiome. Fecal samples were collected from 282 calves: 194 Holstein (H) and 88 Jersey (J). Health status was evaluated daily. Calves with a fecal score $\leq$ 2 and no clinical illness were classified as healthy. Calves with a fecal score $\geq$ 3 were diagnosed with GI disease and classified as bright-sick (BS) or depressed-sick (DS) according to their behavior. A total of 45 samples from healthy calves (n = 38 H, n = 7 J), 11 samples from BS calves (n = 5 H, n = 6 J), and 15 samples from DS calves (n = 6 H, n = 9 J), were randomly selected to represent different breeds, ages (4–15 d of age) and disease severity. The V3-V4 region of 16S rRNA gene was sequenced and an ASV table was used to compare taxonomic profiles. Differences were identified by LEfSe ($P < 0.05$; LDA score > 2). Firmicutes was identified as the most dominant phylum in calves with GI disease, and Actinobacteria in healthy calves. Healthy calves showed abundant *Bifidobacteriaceae*, *Bacteroidaceae* and *Eubacteriaceae*. BS calves showed abundant *Listeriaceae*, *Clostridiaceae* and *Lachnospiraceae*. DS calves showed abundant *Lactobacillaceae*, *Streptococcaceae* and *Enterobacteriaceae*. Specific breed differences included a greater abundance of *Bacteroides fragilis* (LDA = 4.44) in healthy Jersey calves, and *Bifidobacterium longum* (LDA = 5.25) in healthy Holstein calves. *Lactobacillus reuteri_vaginalis* (LDA = 5.02), was abundant in DS Jersey calves, and *Escherichia_Shigella coli* (LDA = 5.01) was abundant in DS Holstein calves. Notably, the abundance of the family *Lactobacillaceae* in diseased calves raises questions regarding its role in sustaining a favorable microbial balance, given that certain lactic acid bacteria have been shown to reduce the incidence of diarrhea in calves and provide protective effects against opportunistic pathogens at the intestinal level.

Key Words: microbiome, calf, diarrhea

230 Implementation of an antimicrobial-use algorithm for treatment of diarrheic calves: Impact on antimicrobial treatment and mortality rates. D. Gomez[1], L. Arroyo[1], D. Renaud*[2], and J. S. Weese[3], [1]*Department of Clinical Studies, Ontario Veterinary College, University of Guelph*, *Guelph, ON, Canada*, [2]*Department of Population Medicine, Ontario Veterinary College, University of Guelph*, *Guelph, ON, Canada*, [3]*Department of Pathobiology, Ontario Veterinary College, University of Guelph*, *Guelph, ON, Canada*.

Calf diarrhea is the major cause of disease and usage of antimicrobial agents in calves <30 d of age on dairy farms. Approaches are needed to reduce, refine and standardize the use of antimicrobial (AB) therapy in diarrheic calves. The objective of this study was to evaluate the impact of implementing an antimicrobial-use algorithm on AB treatment and mortality rates of diarrheic calves. The study consisted of 2 periods: (a) Data analyses of AB treatment and mortality rates of dairy calves from 10 farms (F1- F10) before the implementation of the algorithm (before period, BP; 1 year) and (b) data analyses of AB treatment and mortality rates after implementation of the algorithm (after period, AP; 1 year). The algorithm directed AB therapy based on the presence of fever (≥39.5°C) and hyporexia in calves suffering from diarrhea. Treatment records of 2049 and 2251 calves were available in the BP and AP. Overall, the incidence of diarrhea was similar between periods (BP: 77% (1,573/2,049) vs. AP: 75% (1,698/2,251); $P = 0.316$). Implementation of the algorithm resulted in a marked reduction in antimicrobial treatment rates. In the BP, 85% (1303/1573) of the diarrheic calves were treated with antimicrobials, while in the AP, 18% (310/1698) were treated ($P < 0.001$). Reduction of the antimicrobial treatment rates of diarrheic calves was observed in 7 farms in the AP ($P < 0.01$), but differences were not observed in 3 farms ($P > 0.05$). In general, there were no differences in the overall mortality (BP: 4% (75/2,049) vs. AP: 3% (72/2,251); $P = 0.449$) or the mortality attributable to diarrhea between periods (BP: 1% (22/1,573) vs. AP: 0.6% (12/1,592); $P = 0.73$). The use of user-friendly antimicrobial-use algorithms can reduce significantly the use of antimicrobials with no identifiable negative impact on calf health.

Key Words: calf diarrhea, antimicrobial use, mortality rates

231 Antimicrobial use and decision making with respect to treatment of diarrhea in Canadian dairy calves. T. Uyama*[1], D. Kelton[1], S. LeBlanc[1], D. Léger[2], S. Dufour[3], J. Roy[3], H. Barkema[4], E. de Jong[4], K. McCubbin[4], M. Fonseca[5], L. Heider[5], and D. Renaud[1], [1]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*, [2]*Centre for Food-borne, Environmental & Zoonotic Infectious Diseases, Public Health Agency of Canada*, *Guelph, ON, Canada*, [3]*Faculté de médecine vétérinaire, Université de Montréal*, *St-Hyacinthe, QC, Canada*, [4]*Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary*, *Calgary, AB, Canada*, [5]*Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island*, *Charlottetown, PEI, Canada*.

Antimicrobial resistance in livestock is a growing concern due to possible transmission to humans so it is important to understand antimicrobial use in farm animals. Dairy calves may receive antimicrobials for the treatment of diarrhea, but it is unclear under what circumstances antimicrobials are used. The objective of this study is to investigate antimicrobial use and case-specific information used in treating diarrhea in Canadian dairy calves. A total of 105 dairy farmers (Ontario: 31; Alberta: 28; British Columbia: 26; Nova Scotia: 20) were selected purposively and completed a questionnaire in person about calf health. First, farmers were asked, "Do you use antimicrobials to treat calf diarrhea?" Second, only those who used antimicrobials to treat diarrhea were asked, "What case-specific information do you use to select a diarrhea case for antimicrobial treatment?" Respondents were instructed to select all that apply from a list of 4 symptoms (fecal consistency, fever, attitude, level of dehydration) or otherwise specified. The average herd size was 162 milking cows (range 36–560). Among 105 farmers, 72% used antimicrobials to treat diarrhea. Among those who used antimicrobials for diarrhea, 78% used "fecal consistency," 61% used "fever," 55% used "attitude," 53% used "level of dehydration," and 28% used 11 other characteristics as indicators to treat diarrhea with antimicrobials. The most common answer was selecting all 4 symptoms given, which was selected by 20% of those who used antimicrobials to treat diarrhea. Among 59 farmers who used fecal consistency as an indicator to treat diarrhea with antimicrobials, only 15% solely used this criterion. Among 91 farmers who were asked whether they had a written treatment protocol for calf diarrhea, 35% reported that they have the protocol and 94% of them were discussed with veterinarians. Treatment decisions could be improved for those who solely depend on fecal consistency as an indicator to treat diarrhea with antimicrobials, as well as developing protocols with a veterinarian, to encourage prudent use of antimicrobials in dairy calves.

Key Words: dairy calf, treatment protocol, case-specific information

232 Behavior activity derived from 3-dimensional accelerations to monitor diarrhea in neonatal dairy calves. T. C. Michelot-

Table 1 (Abstr. 233). *Bacillus licheniformis* and *B. subtilis* enzymatic activity profile[1]

Species	Protease	Cellulase	Amylase	Xylanase	Galactomannanase	Galactanase	X-gal
B. licheniformis	—	xx	(x)	(x)	x	(x)	(x)
B. subtilis	x	xx	x	xx	xx	(x)	—

[1] Where (x), x, and xx represent increasing zones of clearance/more enzyme activity; — = none.

ti*[1], L. Drehmer[1,2], A. J. Triminio[1,3], N. A. Carpinelli[1], and J. S. Osorio[1], [1]*South Dakota State University*, *Brookings, SD*, [2]*Universidade Federal de Lavras*, *Lavras, Minas Gerais, Brazil*, [3]*Escuela Agrícola Panamericana El Zamorano*, *El Zamorano, Francisco Morazan, Honduras*.

Diarrhea continues to be the primary cause of morbidity and mortality in preweaned dairy calves. This disease results in economic losses and long-term effects on health and productivity of surviving animals. Hence, monitoring systems that can be correlated with fecal scores in preruminant calves can allow dairy farmers to implement effective response protocols to such disease. Therefore, the objective of this study was to use 3-D accelerations to derive behavior activity (e.g., standing or lying time) and associate these with fecal scores in neonatal dairy calves. Forty-two healthy newborn dairy calves were used from birth until 21 d of age in a retrospective analysis. Calves were housed in individual hutches, had free access to starter and water, and were fed 2.8 L of milk replacer 2 × /d during wk 1 to 3. Calves were fitted with an accelerometer (Onset; Pocasset, MA) mounted in the rear left leg set to record every 60-s throughout the experiment. Fecal score (FS), respiratory score, rectal temperature, and starter intake were recorded daily, while BW and withers height were measured weekly. Overall fecal score (FS) of all calves reached its highest points during wk 2, with a maximum value on 10 d (FS = 2.1 ± 0.1). Based on average individual FS, calves were classified as low FS (LFS; FS = 1.47 ± 0.1; n = 21) and high FS (HFS; FS = 2.14 ± 0.1; n = 21). Data were analyzed using PROC MIXED of SAS with group, time, and their interaction as fixed effects and calf within group as random effect. As expected, FS was greater ($P < 0.01$) in the HFS group during the first 21 d of life. LFS calves showed greater ($P < 0.01$) starter intake (0.23 vs 0.10 kg/d), BW (50.0 vs 44.1 kg), and withers height (82.0 vs 78.8 cm), while lower respiratory score ($P = 0.02$) and rectal temperature ($P < 0.01$) than HFS calves. The HFS calves tended ($P = 0.11$) to have greater lying time than LFS. Although standing bouts were unaffected ($P = 0.64$) by FS, lying bouts tended to be greater ($P = 0.06$) in HFS calves than LFS. Differences in standing and lying behavior indicate that these parameters could be used to monitor diarrhea in neonatal dairy calves.

Key Words: calf scour, accelerometer, diarrhea

233 In vitro evaluation of *Bacillus licheniformis* and *Bacillus subtilis* enzyme activity, *Clostridium perfringens* Type A inhibition, and biofilm formation.

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The use of *Bacillus* as direct-fed microbials (DFM) has been investigated due to their ability to produce antimicrobials and synthetize enzymes. In ruminants, *Bacillus* supplementation has been reported to improve performance and limit diseases. The aim of this study was to investigate (1) the enzymatic activity profile of 2 *Bacillus* strains (*B. licheniformis* [BL], DSM 5749, and *B. subtilis* [BS], DSM 5750), (2) their inhibitory capacity toward *Clostridium perfringens* Type A (CPa, DSM 756), and (3) their biofilm synthesis capacity. Enzyme activity was measured qualitatively with insoluble chromogenic substrates and on milk agar for protease activity. CPa inhibition was evaluated qualitatively by using an agar diffusion assay. In addition, the Ankom RF Gas Production System was used to measure gas production of CPa with or without BL or BS incubated in a Brain Heart Infusion (BHI) broth (37°C, 24h, in triplicate). Biofilm formation was assessed by crystal violet staining after overnight incubation in BHI broth at 37°C. Statistical analysis was performed using the one-way ANOVA with the Dunnett's post-hoc test (GraphPad Prism). Table 1 reports the enzyme activities and shows that *Bacillus* differed in their capacity to produce enzymes. BL and BS were able to generate similar inhibition zones against CPa. The quantitative method also revealed that BL and BS significantly reduced CPa gas production (20%, $P < 0.001$, and 12%, $P < 0.01$, respectively). Finally, BL appeared to be a strong biofilm producer ($OD_{595nm} > 2$) while BS weakly produced biofilm ($OD_{595nm} < 1$). The results show that the enzyme activity, and the ability to produce antimicrobials and biofilm appear to be specific features for each *Bacillus*. In vivo studies need to be conducted to test if BL and BS could enhance ruminant performance and health.

Key Words: *Bacillus*, direct-fed microbial (DFM), ruminant

Production, Management, and the Environment

234 Performance, feed efficiency, and carcass composition of growing beef dairy steers. J. Johnson*, G. Toro, C. Lundgren, A. Arnett, and N. Deeb, *STgenetics*, *Navasota, TX.*

The era of sexed semen and genomics along with changing packer demands has created a perfect opportunity for dairymen to add value to terminal dairy calves by utilizing beef sires on low genomic dairy females. Between 2017 and 2018, domestic sales of dairy semen dropped by more than 1.3M straws while an astonishing 1.5M additional straws of beef semen were sold. Given the impact beef × dairy calves will have on the industry, the objective of this study was to evaluate the performance, feed efficiency, and carcass composition of beef × dairy steers. Performance, DMI, and final ultrasound measurements of back fat depth, LM area, and intramuscular fat were collected on 18 AN × HO and 32 AN × F_1 JE-HO steers during a 70-d period at our research farm in Texas. RFI was calculated from linear regression of DMI on mean $BW^{0.75}$ and ADG. Effects of dam breed and RFI class were evaluated using a mixed model which included the random effect of pen and fixed effects of dam breed, RFI class, and dam breed × RFI class. ADG and DMI ranged from 1.12 to 1.94 kg/d and 7.98 to 12.3 kg/d, averaging 1.55 and 9.83 kg/d, respectively. Steers born to HO dams had higher ($P < 0.05$) initial and final BW, ADG, and DMI than calves of F_1 JE-HO dams, but no differences were observed across dam breeds for feed efficiency or carcass composition traits. As expected, low-RFI steers consumed 11% less feed (9.50 vs. 10.68 kg/d; $P < 0.05$) and had 14% higher ($P < 0.05$) G:F than their high-RFI counterparts with no differences ($P > 0.05$) observed in BW or ADG. Ultrasound measurements did not differ ($P > 0.05$) across RFI classes and no significant dam breed by RFI class interactions were observed. Results from this study provide information regarding the expected performance of beef × dairy calves and demonstrates opportunity to identify elite beef sires based on performance and feed efficiency. The practice of using beef sires on dairy females will not diminish in the near future, however, selection of high-quality sires will become vital for long-term profitability. The data presented is part of an ongoing project to evaluate the performance, feed efficiency, and carcass traits of beef × dairy progeny.

Key Words: beef, dairy, residual feed intake (RFI)

235 Effects of dietary forage level and source on partial carbon footprint of milk in Holsteins and Jerseys. M. E. Uddin*[1,3], H. A. Aguirre-Villegas[2], R. A. Larson[2], and M. A. Wattiaux[1], [1]*Department of Dairy Science, University of Wisconsin-Madison*, *Madison, WI*, [2]*Department of Biological Systems Engineering, University of Wisconsin-Madison*, *Madison, WI*, [3]*Department of Animal Science, University of California-Davis*, *Davis, CA*.

We determined the partial carbon footprint (CF) of milk for 4 diets fed to 2 breed using measured enteric methane and greenhouse gas emissions during manure storage and after field application. Emissions and animal performances were collected in companion studies with diets containing forage neutral detergent fiber at 2 levels (NDF; 19 and 24% of dry matter, referred as low forage and high forage diets, respectively) and from 2 sources [70:30 or 30:70 ratio of alfalfa silage (AS) NDF and corn silage (CS) NDF]. Measured emissions were incorporated in a modeled Wisconsin dairy farm of 117 ha of cropland with a dairy herd consisting of 122 lactating cows (all primiparous), 22 dry cows, and 119 heifers. We assumed that manure was field-applied according to a nutrient management plan, and cropland was used to produce AS, CS and corn grains fed to the cows. Purchased inputs included other concentrate feed to balance rations and chemical fertilizers necessary to fertilize the crops as recommended. The cradle-to-gate life cycle assessment was performed with SimaPro using fat- and protein-corrected milk (FPCM) as the functional unit. Emissions were allocated between milk and meat using either economic or mass allocation. Low forage-fed cows had 11% greater CF than high forage-fed cows (1.57 vs. 1.42 kg CO_2-e/kg FPCM) most likely due to the increase in both DMI and milk production when cows were fed with greater content of highly digestible soyhull in the low-forage compared with the high-forage diets. Forage sources did not influence CF (1.50 kg CO_2-e/kg FPCM). The CF for Holsteins was 5% greater than for Jerseys (1.55 vs. 1.48 kg CO_2-e/kg FPCM). Overall, CF was 1.5 kg CO_2-e/kg FPCM when using economic allocation, but 1.42 kg CO_2-e/kg FPCM when using mass allocation. Under this study conditions, differences in enteric emission intensity across diets were minimal but differences in emission intensity became substantial when assessed by combining the effects of the cow, the manure, the on-farm crop rotation, and the purchased feed, highlighting the need for an integrated approach to assess the diet effects on milk CF.

Key Words: alfalfa silage, corn silage, greenhouse gas

236 Evaluating the establishment of perennial forages with annual warm-season grasses as companion crop on forage yield and quality. S. Thevakumaran*[1], C. Matteau[2], B. Baurhoo[1,2], P. Seguin[1], and A. Mustafa[1], [1]*McGill University*, *Saint Anne de Bellevue, QC, Canada*, [2]*Belisle Solution Nutrition Inc*, *Saint-Mathias-sur-Richelieu, QC, Canada*.

The use of annual warm-season grasses as companion crops in the establishment of perennial forages have not been fully explored. This study evaluated forage yield, chemical composition and in vitro total-tract NDF digestibility (TTNDFD) of perennial mixtures (alfalfa, clover and tall fescue; control) seeded solely or with an annual companion crop [Sudangrass (SG), Sudangrass brown midrib (BMR) gene 12 (BSG), Sorghum-Sudangrass BMR gene 6 (BSSG) or oat]. Experimental plots (8 replicates per treatment) were harvested 60 d (1st cut) and 90 d (2nd cut) after planting at bud stage of alfalfa. The TTNDFD was estimated from potentially digestible NDF (pdNDF), digestion rate (kd) and passage rate (kp) while forage indigestible NDF (iNDF) was calculated from 240 h in vitro incubation. Potentially degradable NDF was calculated by subtracting iNDF from total NDF whereas digestion rate of pdNDF was estimated by in vitro incubation at 24, 30 and 48 h. Data were analyzed as repeated measures using the MIXED procedure of SAS with fixed effects of treatment and cut. Results showed that total forage yield (2 cuts; DM basis) was higher ($P < 0.0001$) for SG (6.8 T/ha), BSSG (6.7 T/ha) and BSG (6.3 T/ha) than control (4.1 T/ha). Oat produced 87% less forage yield in the second cut than first cut. Companion forages reduced ($P < 0.0001$) the yield of perennial legumes and weeds. Relative to control, companion forages reduced ($P < 0.0001$) lignin and crude protein but increased ($P < 0.0001$) NDF and ADF contents of harvested forages. Water-soluble carbohydrate concentration was highest with oat (12.4%), intermediate with SG (9.5%), BSG (9.1%) and BSSG (10.7%) and lowest with control (7.0%). Estimated milk yield was higher ($P < 0.0001$) with companion forages than control. In vitro TTNDFD of second cut followed the order ($P < 0.0001$): BSG and BSSG (average 62.2%) > SG (58.5%) > oat (53.3%) > control (52.3%). The iNDF was lower ($P < 0.0001$) with BSSG than control and oat treatment. In conclusion, establishing perennial forages with SG, BSG or BSSG may improve forage yields, nutritive value and fiber digestibility.

Key Words: cow, forage, fiber digestibility

237 Annual rhythms of feed intake and milk production in the western United States and relationships to management strategies. W. Sanchez[1], K. Johnson[1], K. Harvatine[2], and I. Salfer*[3,2], [1]*Diamond V*, *Cedar Rapids, IA*, [2]*The Pennsylvania State University*, *University Park, PA*, [3]*South Dakota State University*, *Brookings, SD*.

Milk yield (MY) and milk components follow a repeatable yearly cosine function. Generally, in the US MY peaks in mid-April and reaches a nadir in mid-October, whereas milk fat concentration (FAT) and protein concentration (PRO) peak near January 1 and reach nadir near July 1. We have previously demonstrated that these rhythms differ across regions of the US, with MY having greater amplitude in the Southeast and Southwest US, and FAT and PRO having greater amplitude in the Upper Midwest and northeast. However, we have not characterized these rhythms in the Western US, and little is known about how annual rhythms vary among different management systems. This experiment used 129,530 monthly milk production and feed intake records collected from 2006 to 2019 from 332 herds throughout California, the Pacific Northwest, the Southwest, the Rocky Mountains and the Upper Midwest. We examined the effects of region, breed, housing type (freestall vs. loose), and milking frequency (2x/d vs. 3x/d) on annual rhythms of dry matter intake (DMI), MY and FAT and PRO by fitting monthly production records to the linear form of cosine functions with a 12-mo period in SAS 9.4. Dry matter intake fit a 12-mo rhythm in all regions except the Rocky Mountains ($P < 0.01$) and peaked between Feb 24 and Mar 22. The greatest amplitude of the DMI rhythm occurred in the Southwest (2.4 kg). Milk yield fit an annual rhythm in all regions ($P < 0.01$) and had a greater amplitude in the Southwest and California compared with the Pacific Northwest, Rocky Mountains and Upper Midwest ($P < 0.05$). Milk yield fit a 12-mo rhythm regardless of breed ($P < 0.01$), but the amplitude of the rhythm was greater for Holsteins than Jerseys or Crossbreds ($P < 0.05$). Relative to freestalls, cows on loose housing had 22%, 63%, and 57% greater amplitudes of MY, FAT, and PRO rhythms, respectively ($P < 0.05$). Milking frequency did not affect the annual rhythms of MY, FAT, or PRO ($P > 0.10$). In conclusion, milk and components in the Western US follow annual rhythms that are impacted by latitude similar to previous reports, and are also modified by housing system.

Key Words: annual rhythm, milk synthesis, yearly pattern

238 An open-source microprocessor-based sensor for monitoring grazing animal behaviors. B. R. dos Reis*, D. Fuka, Z. Easton, and R. R. White, *Virginia Tech*, *Blacksburg, VA*.

Precision animal agriculture is a rapidly expanding field; however, innovations in this sector are limited by availability of low-cost, low-power sensors that have capacity to send data over long distances without reliance on cellular, bluetooth, or internet-based networking. The objective of this study was to construct an open-source, microprocessor-based sensor designed to detect and report location and activity of pastured ruminants. The sensor comprises an Arduino Nano microprocessor ($4), a generic MPU92/50 motion sensor ($8) which contains a 3-axis accelerometer, 3 -axis magnetometer, and a 3-axis gyroscope, a generic GPS receiver ($5), and a RFM95W generic LoRa radio ($7). The Arduino can be programmed flexibly using the open source Arduino IDE software to adjust the frequency of sampling, the data packet to send, and what conditions are needed to operate. The LoRa radio transmits to a Dragino LoRa gateway ($60) which can also be flexibly programmed through the Arduino IDE software to send data to local storage or, in cases where a web or cellular connection is available, to cloud storage. The sensor was powered using a generic 3.7 V, 2000 mAh Lithium ion battery. The battery, unassisted, was able to power the sensor at a 1 Hz sampling rate for approximately 12 h. For solar assistance, the battery can be connected to a 5 V solar panel ($30) using an Adafruit USB/DC/Solar Lithium Ion/polymer charger ($18). We demonstrated the utility of this sensor suite to timestamp animal location data with motion sensor information by deploying the sensor suite on grazing animals over a 24 h period. For this demonstration, sampling once per 3 min was used. Data recovery rates were excellent (>90%) and all sensors were able to maintain power for the duration of the utility demonstration. Future work should refine the power usage and design of sensors to enable verification of GPS data, training algorithms to interpret motion data, and expansion of the sensor suite.

Key Words: accelerometer, GPS, behavior detection

Reproduction

239 Physiological mechanisms underpinning fertility differences in cows with divergent genetic merit. R. C. Doyle*[1,2], C. Millar[1], S. G. Moore[1], S. A. Holden[1], M. C. Lucy[2], and S. T. Butler[1], [1]*Teagasc*, *Fermoy, Cork, Ireland*, [2]*University of Missouri*, *Columbia, MO.*

The objective was to examine the physiological differences between elite genetic merit Holstein Friesian (EL-HF), average genetic merit HF (NA-HF), Danish Jersey (DKJ) and New Zealand Jersey (NZJ) cows that may underpin differences in fertility. Vaginal discharge score (VDS) exams were carried out weekly on EL-HF (n = 72), NA-HF (n = 36), DKJ (n = 36) and NZJ (n = 36) until a sample with no purulent material was obtained (score = 0). Pre-ovulatory plasma estradiol (E2) and luteal phase plasma progesterone (P4) concentrations were determined in a subset of cows [EL-HF (n = 19); NA-HF (n = 11); DKJ (n = 10), and NZJ (n = 7)] managed under 3 feeding treatments (low grass allowance [LGA; n = 11); control (n = 21); high concentrate (HC; n = 17)] during a synchronized estrous cycle. Postpartum interval until VDS = 0 was recorded and analyzed using survival analysis. Plasma E2 and P4 concentrations were analyzed using a generalized linear mixed-model. An effect of genotype on days to VDS = 0 was observed (38.6, 38.5, 29.64 and 45.4 d for EL-HF, NA-HF, DKJ and NZJ, respectively; $P < 0.0001$). There was a shorter postpartum interval to a VDS = 0 with increasing parity number (44.0, 39.6 and 37.4 d for parity 1, 2 and 3+ respectively; $P < 0.001$), greater fertility index (40.4, 40.6 and 38.6 d for low, medium and high fertility index, respectively; $P < 0.001$) and reduced calving difficulty (39.2 vs. 43.7 d for normal vs. difficult calving; $P < 0.001$). Greater circulating concentrations of P4 were observed in EL-HF compared with NA-HF cows, and in DKJ compared with NZJ cows (genotype effect: $P = 0.03$). The LGA and control feeding treatment cows had greater plasma E2 ($P = 0.03$) and P4 ($P = 0.03$) concentrations than the HC treatment cows. Reproductive phenotypes including VDS and reproductive hormone concentrations were influenced by cow genetic merit, breed, parity, calving difficulty, and feeding regimen and environmental factors.

Key Words: progesterone, estradiol, vaginal discharge score

240 Effect of two different approaches of synchronization of follicular wave emergence on follicle turnover, oocyte recovery and quality, and early in vitro developmental competence of embryos in Nili-Ravi buffaloes. M. Sagheer[1], F. Ullah[1], M. Saleem[1], M. Nawaz[1], A. Riaz[1], U. Arshad*[2], and N. Ahmad[1], [1]*Department of Theriogenology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences*, *Lahore, Pakistan*, [2]*Department of Animal Sciences, University of Florida*, *Gainesville, FL.*

The objective of the present study was to evaluate the effect of 2 methods of follicular wave emergence on follicle turn-over, oocyte recovery (OR), quality of the oocytes (OQ), and early in vitro developmental competence of embryos (IVEP) in Nili-Ravi buffaloes. In a 2 × 2 crossover design, buffaloes (n = 8) were randomly divided to receive either 1) FA [follicular ablation; n = 4], or 2) CIDR-EB [synchronization protocol, n = 4]. In FA group, the ovarian follicles were aspirated (week −1) and subsequently repeated OPU sessions (n = 55) were performed for 7 weeks. However, in CIDR-EB treated buffaloes, a P4 device (CIDR) inserted in the anterior vagina and a single dose of EB (2 mg) and prostaglandin (150 µg) were administered (wk 1). The CIDR was removed on d 7 and repeated OPU sessions (n = 56) were performed. In both groups, a 7-d resting period was provided between each OPU session. Data on the number of follicles, OR, OQ, and IVEP were analyzed using the PROC MIXED and GLIMMIX procedures of SAS. Results (LSM ± SEM) revealed that the number of small (3.50 ± 0.63 vs.2.69 ± 0.60) sized follicles was higher ($P < 0.05$) in FA-treated, whereas, medium (1.13 ± 0.45 vs. 1.59 ± 0.45) sized follicles were higher ($P < 0.05$) in CIDR-EB treated buffaloes. However, the large (0.04 ± 0.01 *vs*. 0.02 ± 0.01) sized and total number of follicles (4.65 ± 0.88 vs. 4.42 ± 0.88) did not differ ($P > 0.30$) across the treatments. The OR (0.67 ± 0.18 vs. 0.53 ± 0.20), and OQ (Grade I+II; 0.15 ± 0.09 vs. 0.17 ± 0.09 and grade III+IV; 0.85 ± 0.02 vs. 0.83 ± 0.02), remained similar ($P > 0.05$) in both groups, respectively. Similarly, rates of in vitro maturation (0.66 ± 0.18 vs. 0.59 ± 0.20), cleavage (0.42 ± 0.19 *vs*. 0.52 ± 0.20), and morula (0.13 ± 0.13 vs. 0.08 ± 0.10) did not differ ($P > 0.05$) between the groups, respectively. Taken together, CIDR-EB enhances the medium-sized follicles, however, the 2 fundamental approaches of synchronization of wave emergence result in similar efficiency for early IVEP in buffaloes.

Key Words: follicular wave emergence, in vitro developmental competence of embryos, buffalo

241 Evaluation and characterization of estrus alerts from an ear-attached accelerometer-based automated estrus detection system. E. M. Schilkowsky, G. E. Granados, E. M. Sitko, M. Masello, M. M. Perez, and J. O. Giordano*, *Cornell University*, *Ithaca, NY.*

Our objectives were to evaluate an ear-attached automated estrus detection system (AEDS; Smartbow; Zoetis Inc.) that monitored physical activity (PHA) and rumination time (RUM) for detection of estrus and characterize AEDS estrus alert features. Lactating Holstein cows (n = 216) had their estrous cycle synchronized at 50 ± 3 DIM or 18 ± 3 d after AI. For 7 d after induction of luteolysis with $PGF_{2\alpha}$ (d 0) visual observation of estrus behavior (30 min 2 times per day) and data from a pressure-activated tail-head sensor (HeatWatch) were used as reference test (RT) for estrus. Concomitantly, estrus alerts and their features were collected from the AEDS. Progesterone levels confirmed luteal regression and ultrasonography timing of ovulation. Performance metrics for the AEDS were obtained with PROC FREQ of SAS using only estrus detected by the RT or detection of estrus by the RT and ovulation for cows with a discrepancy between the RT and AEDS. Based on the RT, 87% presented estrus and ovulated, 1% presented estrus and did not ovulate, 6% did not present estrus but ovulated, and 6% did not present estrus nor ovulation. Sensitivity (Se), specificity (Sp), negative predictive value (NPV), positive predictive value (PPV), and balanced accuracy (BA) were 92 (88–96), 69 (51–87), 53 (36–70), 96 (93–99), and 80% (70–92), respectively when only the RT was used. After correcting for ovulation for cows with a discrepancy between the RT and AEDS, Se, Sp, NPV, PPV, and BA (mean and 95% CI) were 93 (88–96), 92 (62–100), 44 (32–57), 99 (96–100), and 93% (75–98), respectively. The mean ± SD and range for the interval from induction of luteolysis to estrus alerts, estrus alert duration, and the onset of estrus alerts to ovulation interval were: 72.2 ± 18.1 (30–160), 13.5 ± 3.8 (2–28), and 23.8 ± 7.1 (4–44) h. Cows spent 8.5 ± 18.1 (2–28) min per hour in high active time (measure of estrus intensity). We concluded that an ear-attached AEDS that monitored PHA and RUM was effective at detecting cows in estrus and generated few false positive alerts when accounting for ovulation.

Key Words: ear-attached sensor, estrus, cow

242 Effect of a high dose of gonadorelin hydrochloride at the first gonadotropin-releasing hormone of the breeding-Ovsynch of a fertility program on ovulation rate and pregnancies per AI in first-service lactating Holstein cows. T. Valdes Arciniega*[1], I. M. R. Leão[1], E. Anta Galvan[1], T. O. Cunha[1], M. S. El Azzi[1,2], N. B. Cook[1], and J. P. N. Martins[1], [1]*School of Veterinary Medicine, University of Wisconsin-Madison*, *Madison, WI*, [2]*Universidade Federal de Lavras*, *Lavras, MG, Brazil.*

The aim of the present study was to determine if a high dose of gonadorelin hydrochloride (GnRH, 200 µg; Factrel, Zoetis) i.m. would increase ovulation rate compared with a low dose (100 µg) i.m. at the first GnRH of the breeding-Ovsynch (G1), and thereby increase first service pregnancies per AI (P/AI) in lactating Holstein cows. Weekly cohorts of cows (n = 765) from a commercial dairy farm were randomly assigned according to ending ear tag number (odd or even) to receive either a low or a high dose of GnRH at G1 of the Double-Ovsynch program (GnRH – 7d – PGF – 3d – GnRH – 7d – GnRH (G1) – 7d – PGF – 1d – PGF – 32h – GnRH – 16h – TAI). Primiparous (n = 240) and multiparous (n = 525) cows received treatments at 72 ± 3 and 62 ± 3 DIM, respectively. Ovulation was determined by the disappearance of a follicle with diameter = 9 mm, and the appearance of a luteal structure between the d of treatment and 2 d later by ultrasound. Cows received timed-AI using either conventional Angus (n = 124) or Holstein (n = 468) semen. Pregnancy diagnosis was performed by farm veterinarians using ultrasound at 32 d post-AI. Data were analyzed by logistic regression using Proc GLIMMIX of SAS. The model included fixed effects of treatment, parity and the interaction of treatment × parity. High dose increased ($P < 0.01$) ovulation rates in both primiparous (78.6 vs. 61.9%) and multiparous (84.8 vs. 70.5%) cows compared with the low dose treatment. Multiparous cows also had greater ($P = 0.03$) overall ovulation rate than primiparous cows (77.5 vs. 71.2%). The high dose did not have a significant effect on P/AI in primiparous (64.9 vs. 55.4%; $P = 0.27$) and in multiparous (47.1 vs. 44.8%; $P = 0.62$) cows, compared with the low dose treatment. Overall P/AI was greater for primiparous compared with multiparous cows (60.8 vs. 45.9%; $P < 0.01$). In summary, increasing the dose of GnRH treatment at G1 of the Double-Ovsynch program increased ovulation rate, but it did not significantly improve P/AI in first service lactating Holstein cows, based on preliminary data.

Key Words: GnRH, ovulation, Double-Ovsynch

243 Association between age at first calving and reproductive performance of primiparous cows managed with a program that favored insemination at detected estrus. E. Sitko*, M. M. Pérez, M. Masello, G. E. Granados, and J. O. Giordano, *Department of Animal Science, Cornell University*, *Ithaca, NY.*

Our objective was to evaluate the effect of age at first calving (AFC) on reproductive performance and herd exit of primiparous Holstein cows submitted to AI with a program that favored insemination at detected estrus (AIE). Within farm (n = 5), cows (n = 1,163) were retrospectively grouped by AFC in mo (n; mean; range): low (n = 373; 21.4; 20.3–22.4), medium (n = 421; 22.1; 21.3–23.3), and high (n = 369, 23.5; 21.9–29.7). Cows were eligible for AIE after a PGF2α (PGF) treatment at 53 ± 3 DIM. Cows not AIE by 74 ± 3 DIM received timed AI after GnRH, 7 d PGF, 1 d PGF, 32 h GnRH, 18 h TAI with progesterone supplementation from the first GnRH to PGF. Thereafter, cows were AIE up to the time of nonpregnancy diagnosis at 32 ± 3 d after AI. Nonpregnant cows received the same protocol for TAI than for first AI. Binomial data were analyzed by logistic regression. Time to pregnancy and herd exit up to 200 DIM by Cox's proportional hazard regression. AFC group (AFCG) was included as fixed and farm as random effect. Confounders included season (cold vs. warm), AI type (TAI vs. AIE), milk production group (MLKG; low, medium, and high), and daughter pregnancy rate group (DPRG; low, medium, and high). The proportion of cows AIE for first AI, was affected by DPRG and AFCG ($P < 0.05$), whereby it was greater for low (76.0%) and medium AFC (73.9%) than for high AFC (67.0%). First AI pregnancy per AI (P/AI), was affected by DPRG and the interaction between AI type and AFCG ($P < 0.05$). Within high AFC, P/AI was greater for TAI (59.2%) than for AIE (39.3%), whereas it was similar within medium and low AFC groups. Time to pregnancy was affected by DPRG ($P < 0.05$) but not AFCG ($P > 0.05$). The proportion of cows pregnant up to 200 DIM was not affected by AFCG ($P > 0.05$). MLKG ($P < 0.01$) but not AFCG ($P > 0.05$) affected the hazard of herd exit up to 200 DIM. In conclusion, AFC affected the proportion of primiparous cows AIE for first AI and first service P/AI, but did not affect the pregnancy and herd exit dynamics up to 200 DIM for cows managed with a program that favored insemination at detected estrus rather than TAI.

Key Words: first calving, fertility, estrus

244 Age at first calving affected reproductive performance of primiparous cows managed with a program that favored timed artificial insemination. E. Sitko*, M. M. Pérez, M. Masello, G. E. Granados, and J. O. Giordano, *Department of Animal Science, Cornell University*, *Ithaca, NY.*

Our objective was to evaluate the effect of age at first calving (AFC) on reproductive performance and herd exit of primiparous lactating Holstein cows submitted for AI with a program that favored timed AI (TAI). Within farm (n = 5), cows (n = 1,072) were retrospectively grouped by AFC in mo (n; mean; range): low (n = 323; 21.5; 18.6–22.4), medium (n = 394; 22.1; 21.3–23.3), and high (n = 355, 23.5; 21.8–29.6). AFC differences were the outcome of different reproductive performance as heifers. First service TAI was at 84 ± 3 DIM after Double-Ovsynch [GnRH, 7 d $PGF_{2\alpha}$ (PGF), 3 d GnRH, 7 d GnRH, 7 d PGF, 1 d PGF, 32 h GnRH, 18 h TAI]. Thereafter, cows received AI at detected estrus (AIE) any time or TAI if not AIE up to the time of nonpregnancy diagnosis at 32 ± 3 d after AI. Nonpregnant cows with a corpus luteum (CL) received: GnRH 25 ± 3 d after AI, 7 d PGF, 1 d PGF, 32 h GnRH, 18 h TAI. Cows with NoCL received GnRH 25 ± 3 and 32 ± 3 d after AI, 7 d PGF, 1 d PGF, 32 h GnRH, 18 h TAI with progesterone supplementation. Binomial data were analyzed by logistic regression. Time to pregnancy and herd exit up to 200 DIM were analyzed by Cox's regression. AFC group (AFCG) was included as fixed and farm as random effect. Confounders included season (cold vs. warm), milk production group (MLKG; low, medium, and high), and daughter pregnancy rate group (DPRG; low, medium, and high). First AI P/AI, was affected by DPRG ($P < 0.01$), but not AFCG ($P > 0.05$). Time to pregnancy was affected by DPRG ($P < 0.05$) and tended to be affected by AFCG ($P = 0.06$), whereby the hazard of pregnancy (HR; 95% CI) was greater for low than for high (1.21; 1.04–1.42) AFC, and for medium than high AFC (1.12; 1.01–1.25) but was similar for low and medium AFC (1.01; 0.91–1.12). More ($P < 0.05$) cows were pregnant by 200 DIM in the low (93.5%) than high (87.9%) AFC group, whereas the medium (91.6%) group was intermediate. MLKG ($P < 0.01$) but not AFCG ($P > 0.05$) affected the hazard of herd exit. We conclude that AFC affected the reproductive performance of primiparous lactating Holstein cows managed with a program that favored TAI because cows with reduced AFC had improved reproductive performance. Also, AFC did not affect the herd exit dynamics.

Key Words: age at first calving, fertility, timed AI

245 Effect of timing of AI using sexed semen relative to induction of ovulation after a Double-Ovsynch protocol on pregnancy outcomes and fetal sex in lactating primiparous Holstein cows. M. R. Lauber*[1], B. McMullen[3], J. J. Parrish[2], and P. M. Fricke[1], [1]*Department of Dairy Science, University of Wisconsin-Madison*, *Madison, WI*, [2]*Department of Animal Science, University of Wisconsin-Madison*, *Madison, WI*, [3]*Bridgewater Dairy Group*, *Montpelier, OH.*

Our hypothesis was that delaying timing of AI using sexed semen relative to induction of ovulation after a Double-Ovsynch (DO) protocol for first timed artificial insemination (TAI) in lactating Holstein cows would increase pregnancies per AI (P/AI). Primiparous lactating Holstein cows from 3 US dairy farms were randomly assigned to receive a standard DO protocol with the second GnRH treatment (G2) of the breeding Ovsynch portion of the DO protocol administered 16 h before TAI (G2–16; n = 357) or a modified DO protocol with G2 administered 24 h before TAI (G2–24; n = 341). Based on an expected ovulation time between 24 to 32 h after G2, TAI for G2–16 cows occurred approximately 8 to 16 h before ovulation, whereas TAI for G2–24 cows occurred approximately 0 to 8 h before ovulation. Within each herd, cows received TAI using sexed semen (ABS Sexcel Sexed Genetics, DeForest, WI) from sires of their choosing. Sires were randomly allocated between treatments within each herd, and AI technicians were blind to treatment at TAI. Pregnancy status was assessed using transrectal ultrasonography at 34 ± 3 d and 84 ± 20 d after TAI, and fetal sex was determined on 2 of the 3 farms at 69 ± 3 d after TAI (n = 94 and 78 cows for G2–16 and G2–24 cows, respectively). Binary response data were analyzed by logistic regression using the GLIMMIX procedure of SAS with farm included as a random effect in the model, and fetal sex ratio was analyzed by Fisher's Exact test. Contrary to our hypothesis, G2–16 cows had more P/AI than G2–24 cows at 34 ± 3 d (49% vs. 43%; $P = 0.06$) and 84 ± 20 d (47% vs. 40%; $P = 0.03$) after TAI. Pregnancy loss was 5% for both treatments, and fetal sex ratio did not differ between treatments (91% and 88% females for G2–16 and G2–24 cows, respectively). In conclusion, we reject our initial hypothesis; rather, delaying timing of AI using sexed semen relative to induction of ovulation after a DO protocol decreased P/AI in primiparous lactating Holstein cows.

Key Words: sexed semen, dairy cow, timing of AI

Ruminant Nutrition: General

246 Effect of water status (drought) on in situ starch digestibility of corn for silage.

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The objective of this study was to determine the effect of irrigation on in situ starch digestibility (ISSD) of corn for silage. The study was performed in Kimberly (ID) during the 2018 growing season. Five commercial corn hybrids were planted in a split-plot within a randomized complete block design with 4 blocks. Experimental treatments consisted of a control treatment with furrow irrigation at planting and 3 more times during crop growth (watered, W) and a non-irrigated treatment with furrow irrigation only at planting (drought, D). When the corn was between 1/4 and 3/4 milk-line stage of maturity, ears from 3 plants were harvested from each plot and shelled by hand. After drying (55°C) to constant weight, kernels were ground to pass through a 4-mm screen of a cutter mill and placed in porous Dacron bags (3.6 g DM/bag). At 9:30 a.m. of the test day, bags were immersed within the rumen of 3 cannulated cows fed a totally mixed ration (40% corn silage, 7% alfalfa hay, and 53% concentrate mix; DM basis) and incubated for 0, 4, 8, 12, 24, and 48 h. Once dried, all bags were weighed and opened, and the residues were re-ground to pass through a 1-mm screen of a cyclone mill before starch analysis. In situ starch disappearance parameters were determined using NLIN procedure of SAS according to the model $\mathrm{ISSD} = A + B \times [1\text{-}e^{(-k \times T)}]$, where A is the digested starch at time (T) zero, B is the potentially digested starch, and k is the fractional starch digestion rate. Undigestible starch (C) was also determined. Kinetic parameters were contrasted using a mixed model that included block (random; 3 df), treatment (fixed; 1 df), block × treatment (random; 3 df), hybrid (fixed; 4 df), treatment × hybrid (fixed; 4 df), and the random residual error. Whole-plant dry matter concentration was greater for D corn than for W corn (36.9 vs. 30.3%; $P < 0.01$). Water status did not affect fractions A (20.3%; $P = 0.16$), B (75.9%; $P = 0.36$), or C (3.8%; $P = 0.19$), the fractional starch digestion rate (6.7%/h; $P = 0.96$), or the half-life of starch (7.4 h; $P = 0.21$). In conclusion, under the conditions of this study, water stress had no effects on in situ starch digestibility of corn for silage.

Key Words: drought stress, corn silage, starch digestibility

247 Simulating the effect of two-day-delayed sealing on the fermentation characteristics and aerobic stability of an alfalfa crop during the early stage of fermentation, treated with a dual strain inoculant.

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Adverse event during silage making, such as delayed sealing, could influence silage quality and resulting aerobic stability, when fermented for short time. The aims was to study the effects of a silage inoculant on fermentation and aerobic stability (AS) of alfalfa silage evaluated after 14 d of fermentation, in normal ensiling conditions (NORM) or in a challenge situation (DELAY = 2 d delayed sealing and forage sprinkled with water to simulate 7 mm of rainfall). To test this hypothesis, 3 20 l mini-silos/treatment were filled with wilted alfalfa (183 ± 4.6 kg DM/m^3, 39.6% DM NORM or 35.5% DM DELAY). For both NORM and DELAY, forage was treated with no inoculant (CTR) or with SiloSolve FC (FC) containing 50:50 of *Lactobacillus buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037). The target application rate was 150,000 cfu/g of forage. The mini-silos were stored for 14 d at 20°C, meaning the DELAY setting fermented anaerobically for only 12 d. After fermentation, each mini-silo followed an AS test by monitoring the temperature (T) increase inside the mini-silos until they reached +3°C above T ambient. The statistical model examined the fixed effect of ensiling conditions (NORM vs. DELAY), inoculation (CTR vs. FC), and their interaction. The pH increased (4.55 vs. 5.34, $P < 0.05$) and lactate decreased (3.66 vs. 1.56% DM, $P < 0.05$) in NORM and DELAY silages, respectively. Use of SiloSolve FC numerically enhanced acetate (2.46 in CTR vs. 3.11% DM in FC, $P = 0.16$), whereas yeast count was decreased (5.1 vs. 3.9 $\log_{10}$ cfu/g, respectively in CTR and FC; $P < 0.05$). AS in NORM treatment was increased by 6 d in FC silages compared with CTR ($P < 0.05$). However, the DELAY reduced AS in both CTR and FC (13 vs. 26 h; $P = 0.715$). These results indicated that use of SiloSolve FC improves aerobic stability and fermentation even after a very short period of fermentation in the NORM group. In the DELAY, the short fermentation time was insufficient to obtain a stable silage, despite a numerical improvement of FC over CTR.

Key Words: aerobic stability, *Lactobacillus buchneri*, *Lactococcus lactis*

248 Assessing the antifungal activity of various sources of sodium lignosulfonate and chitosan against fungi isolated from spoiled hay.

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Our objective was to assess the antifungal activity of a collection of 4 sodium lignosulfonate [Sappi (NaSP), Sigma-Aldrich (NaAL), Beantown (NaBT), and Spectrum (NaUM)] and 2 chitosan [naive (ChNv) and microparticles (ChMp)] sources. We determined their minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) against *Aspergillus amoenus*, *Mucor circinelloides*, *Penicillium solitum*, and *Debaromyces hansenii* at pH 4 and 6 using malt-extract broth and agar. Propionic acid (PRP) was the positive control. The MIC and MFC assays were carried out in duplicate in each of 3 independent runs. Across lignosulfonates, NaSP was the most effective inhibitor and fungicide at pH 4 (Table 1), except for *M. circinelloides*. At both pH 4 and 6, PRP was more antifungal than NaSP, which had no activity at pH 6. Except for *M. circinelloides*, ChNV and ChMp were more effective at killing than lignosulfonates and PRP at both pH 4 and 6. In conclusion, NaSP was the most antifungal lignosulfonate tested. Despite not inhibiting all fungi tested, chitosans had the strongest antifungal activity when effective. Results warrant their development as potential hay preservatives.

Key Words: spoilage, lignosulfonates, chitosan

249 Effect of chemical and biological preservatives on the dry matter loss, nutritional composition, microbial counts, and aerobic stability of ensiled wet brewer's grain.

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This study evaluated the effects of a wide array of preservatives on spoilage of wet brewer's grain (WBG) silage. Treatments (TRT) were sodium lignosulfonate at 1% (NaL1) and 2% (NaL2; wt/wt of fresh WBG), propionic acid (PRP; 0.5% wt/wt of fresh WBG), a combination inoculant (INO; *Lactococcus lactis* and *Lactobacillus buchneri* each at 4.9 log cfu/

Table 1 (Abstr. 248). Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values (mg/mL ± SD) of compounds tested against fungi isolated from spoiled hay as a function of media pH

		A. amoenus		*P. solitum*		*M. circinelloides*		*D. hansenii*	
Compound	pH	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
NaSP	4	16.0±4.2	29.0±2.2	15.0±0.0	31.0±5.5	15.0±0	>60	13.8±2.5	13.8±2.5
	6	>60	n.c.[1]	>60	n.c.	>60	n.c.	>60	n.c.
NaAL	4	n.d.[2]	>60	n.d.	>60	n.d.	>60	n.d.	20.0±0
	6	>60	n.c.	>60	n.c.	>60	n.c.	>60	n.c.
NaUM	4	>60	n.c.	>60	n.c.	>60	n.c.	58.3±2.9	>60
	6	>60	n.c.	>60	n.c.	>60	n.c.	>60	n.c.
NaBT	4	n.d.	38.6±2.4	n.d.	40.0±0	n.d.	45.0±0	n.d.	28.8±2.5
	6	>60	n.c.	>60	n.c.	>60	n.c.	>60	n.c.
ChMp	4	n.d.	5.1±1.1	n.d.	1.6±0.9	>8	n.c.	0.07±0.02	0.16±0.0
	6	n.d.	2.9±0.7	n.d.	0.06±0.02	>8	n.c.	0.08±0.0	0.67±0.28
ChNv	4	n.d.	1.9±0.7	n.d.	2.3±0.9	>10	n.c.	0.02±0.0	0.04±0.0
	6	n.d.	1.1±0.7	n.d.	2.2±0.6	>10	n.c.	0.02±0.0	0.07±0.02
PRP	4	1.25±0.0	7.5±2.0	2.5±0.0	5.0±0.0	3.3±0.0	18.8±2.5	1.25±0.0	2.5±0.0
	6	12.0±2.7	>60	16.3±4.8	>60	12.5±2.9	>60	14.0±2.2	15.0±0.0

[1] n.c. = not calculated.

[2] n.d. = cannot be determined visually.

fresh WBG g), and untreated (CON). Five batches of unroasted WBG were collected from the same source right after lautering and cooled to room temperature for 1 d before treatments were randomly applied to each batch. Treated WBG were packed into 8.8 L mini-silos and stored for 60 d at 21°C. Data were analyzed as a randomized complete block design (5 blocks) and differences were declared at $P \leq 0.05$. At d 0, no differences were observed for sugars (~28.8 ± 1.88% of DM) and yeast counts (~2.89 ± 0.346 log cfu/fresh g) across TRT, but NaL2 and NaL1 had a higher DM (~23.4) relative to CON (21.2 ± 0.21%). PRP had a lower pH (4.63) and lactic acid bacteria counts (LAB; 4.50) relative to the other TRT (~6.13 ± 0.024 and ~5.65 ± 0.229 log cfu/fresh g). At opening (d 60), PRP had a lower DM loss (5.06) and a markedly better preservation of sugars (25.0) relative to all other TRT (~10.5 ± 1.26% and ~13.6 ± 1.36% of DM). However, PRP decreased LAB (6.74) relative to the other preservatives (~7.94), except CON (7.27 ± 0.201 log cfu/fresh g). CON and INO had a higher NDF (~47.7%DM) than the other treatments (~41.2 ± 0.77), and lower DM (~21.7) than PRP (23.6 ± 0.33%). CP was lower for PRP, NaL1, and NaL2 (~24.5 ± 0.42% of DM) compared with CON (27.4), and NH$_3$-N (% of N) was higher for PRP and INO (~0.84) relative to other TRT (~0.50 ± 0.057). No major differences were observed for yeast (~4.27 ± 0.366) and mold (<2 log cfu/fresh g) counts. Aerobic stability (h) was greater for NaL2 (103.2) relative to PRP (61.9) but no differences were observed vs. CON (90.1 ± 8.86). In conclusion, PRP preserved WBG nutrients during storage to the greatest extent, compared with the other preservatives tested.

Key Words: wet brewer's grain, silage, preservatives

250 An optimized lignosulfonate-based product matched propionic acid preservation effects on high-moisture alfalfa hay. A. Y. Leon-Tinoco*[1], S. L. Annis[1], S. T. R. Almeida[2], B. C. Guimarães[2], R. Hollandsworth[1], M. Killerby[1], C. Wu[3], R. Kersbergen[1], A. Lichtenwalner[1], B. Perkins[1], C. Knight[1], D. Skonberg[1], Z. X. Ma[4], and J. J. Romero[1], [1]*University of Maine*, *Orono, ME*, [2]*University of Lavras*, *Lavras, MG, Brazil*, [3]*University of Delaware*, *Newark, DE*, [4]*University of Florida*, *Gainesville, FL.*

Our objective was to evaluate the effects of an optimized lignosulfonate-based product (LST, UMaine), chitosan (ChNv, Sigma-Aldrich), and propionic acid (PRP, 99%; MP Biomedicals) on high-moisture alfalfa hay spoilage. Treatments were the factorial combination of 3 preservatives (LST, ChNv, and PRP) and 5 concentrations (0, 0.25, 0.5, 1, and 2% wt/wt fresh basis). Preservatives were added to 35 fresh g of sterile alfalfa hay (71.5 ± 0.23% DM), inoculated with a mixture of previously isolated spoilage fungi (5.27 log cfu/fresh g), and aerobically incubated in vitro for 23 d (25°C). Data were analyzed as a randomized complete block design replicated 5 times (5 independent plots from an alfalfa field) and differences were declared at $P \leq 0.05$. At d 0, the untreated hay had 43.9 ± 2.48, 19.7 ± 1.65, and 11.3 ± 0.17 of NDF, CP, and ash (% of DM), respectively. At d 23, relative to untreated (24.0 ± 0.45%), DM losses were greatly lessened by doses as low as 0.25% for both LST and PRP (~1.61). This was explained by reduced mold counts for as low as 1% LST (<2.0 log cfu/fresh g) and as low as 0.5% PRP (<2.0) vs untreated (6.76). Yeast counts were reduced by as low as 1% LST (<2.0) and as low as 0.25% PRP (<2.0) vs. untreated (6.10). Both as low as 0.25% LST and PRP prevented the decrease in DM (~75.5), and increase in ash (~12.1) and CP (~20.6) relative to untreated (~62.5 ± 0.48%, 15.3 ± 0.12 and 24.1 ± 0.27% of DM). NH$_3$N was decreased by as low as 0.25% for both LST and PRP (~1.11 ± 0.190% of N) vs. untreated (7.80). Consequently, as low as 0.25% for both LST and PRP (~5.37 ± 0.035) prevented the increase in pH observed in untreated (8.89). Increasing the dose of ChNv did not have an effect on DM loss (~23.2 ± 0.45%), mold counts (~6.59 ± 0.440), yeast counts (~6.16 ± 0.368), pH (~8.88 ± 0.035), or DM (~63.5 ± 0.48%) relative to untreated. In conclusion, LST and PRP prevented high-moisture hay spoilage to the same extent, within the doses tested, while ChNV did not relative to untreated.

Key Words: hay preservation, lignosulfonate, chitosan

251 Meta-analysis of the effects of preservatives on hay spoilage I: Chemical treatments. M. Killerby*[1], R. White[2], D. C. Reyes[1], A. Y. Leon-Tinoco[1], S. Rivera[1], H. Paz[3], J. A. Jendza[4], and J. J. Romero[1], [1]*Animal and Veterinary Sciences, School of Food and Agriculture, University of Maine*, *Orono*, [2]*Animal and Poultry Sciences, Virginia Polytechnic Institute and State University*, *Blacksburg, VA*, [3]*Department of Animal and Dairy Scienc-*

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When producers are forced to bale hay above 20% moisture, large losses of DM and nutritive value, and the presence of mycotoxins can be expected. Our objective was to conduct a meta-analysis to evaluate the effects of preservatives on hay spoilage during storage. A literature search was conducted using the ISI Web of Science database where a total of 459 peer-reviewed papers were retrieved. For inclusion in the analysis, studies had to (1) be published in English language peer-reviewed journals; (2) concurrently examine untreated and treated groups; and (3) report the preservative application rate. Chemical preservatives were analyzed as a separate subset from microbial inoculants due to incompatibilities in application rate units (% vs. log cfu/fresh g, respectively). The chemical subset included 330 treatments from 50 articles. The metafor package of R statistical software was used to fit a multilevel linear mixed-effects model with response variable reflecting the standardized mean difference between treated and untreated for DM loss, sugars, and mold presence in hay samples within an experiment, within a study. Experiment and study were used as random effects. Moderators included forage type (FT: grass, legume, or mix); moisture class (MC: below 20% or above 20%); normalized application rate (AR); and preservative classification (PC). The 2- and 3-way interactions among these moderators were also tested. Final models were selected using a backward selection procedure where non-significant variables were removed iteratively if they were not involved in significant interactions. The final model suggested DM losses were significantly related to the interactions between FT and MC (P = 0.044) and between PC and AR (P < 0.001); and to the main effects of FT (P = 0.001), AR (P = 0.050), and PC (P = 0.004). A non-significant main effect for MC (P = 0.355) was also included in the model. Sugar concentration was affected by the 3-way interaction between PC, AR, and MC (P = 0.032); and by forage type (P < 0.001). Mold presence was affected by the 3-way interaction between PC, AR, and MC (P = 0.0267) and by the 3-way interaction between PC, AR, and FT (P < 0.001).

Key Words: hay, chemical preservatives, meta-analysis

Ruminant Nutrition: Carbohydrates and Lipids

252 A hybrid hepatic tissue model to highlight changes in peroxisome-proliferator activated receptor activity through the peripartum period. S. Busato*[1], A. M. Abdelatty[2,1], and M. Bionaz[1], [1]*Oregon State University*, *Corvallis, OR*, [2]*Cairo University*, *Cairo, Egypt*.

The transition from pregnancy to lactation imposes a large degree of metabolic stress on dairy cows, when the overall negative energy balance causes lipolysis and release of fat in the form of nonesterified fatty acids (NEFA). In prior studies, we proved that circulating NEFA activate the peroxisome-proliferator activated receptors (PPAR) in a hybrid in vitro-in vivo model, where immortalized cells were treated with serum at different NEFA concentrations. PPAR are involved in the metabolism of fatty acids and triglycerides (TAG), as well as in the regulation of insulin sensitivity, all crucial processes in the peripartum. Our objective was to assess the role of PPAR activation in the transition period through an ex vivo bovine liver model. Our hypothesis was that activation of PPAR, measurable through changes in gene expression, would be modulated by serum NEFA through the peripartum. Four primiparous Jersey cows received trocar biopsies at −10 and + 10 DIM, and the tissue was sectioned using a Krumdieck Tissue Slicer to obtain thin slices (PCLS) suitable for culture. Two PCLS were then incubated with each treatment: NEFA isolated from serum, as well as whole serum, and isotype-specific synthetic agonists and antagonists of PPAR, applied individually. Transcription of PPAR target genes was measured via RTqPCR, and analyzed through PROC MIXED of SAS 9.4, comparing treatment and DIM effect, and their interaction. Despite large variation between animals, expression of *PPARA, PPARG* and *FABP1* decreased while transcription of PDK4 increased from −10 to +10 DIM. Treatment with a PPARγ agonist increased expression of *ACADVL* and *LIPC* at +10 DIM. NEFA and a PPARδ antagonist decreased *PDK4* expression, and C 16:0 upregulated transcription of *FABP1*. Whole-transcriptome sequencing revealed that treatment of PCLS with PPAR agonists in the postpartum modulates the expression of genes involved in lipid metabolism, as well as amino and nucleic acid metabolism. Despite the changes in gene expression, no change was observed in the TAG concentration in response to PPAR activation. Our findings suggest that our PCLS model is informative at a molecular level.

Key Words: nutrigenomics, peripartum, peroxisome-proliferator activated receptors (PPAR)

253 Effects of serine palmitoyltransferase inhibition by myriocin in ad libitum-fed and nutrient-restricted non-lactating ewes. A. N. Davis*[1], W. A. Myers[1], J. E. Rico[1], L. F. Wang[1,2], C. Chang[1], A. T. Richards[1], M. Moniruzzaman[3], N. J. Haughey[3], and J. W. McFadden[1], [1]*Cornell University*, *Ithaca, NY*, [2]*Henan Agricultural University*, *Zhengzhou, China*, [3]*Johns Hopkins University*, *Baltimore, MD*.

The fungal isolate myriocin is an inhibitor of serine palmitoyltransferase and de novo ceramide (Cer) synthesis in non-ruminants. Our objectives were to determine whether myriocin suppresses de novo Cer synthesis in ruminants. Twelve mature, nonlactating crossbred ewes (49.4 ± 4.90 kg) received an intravenous bolus of ethanol (CON) or 1 of 3 doses of myriocin (0.1, 0.3, or 1.0 mg/kg/BW in ethanol; LOW, MOD, or HIGH) every 48 h for 17 d. Ewes were ad libitum fed a high-energy pelleted and grass hay diet from d 1–14, and nutrient-restricted (straw only) from d 15–17. Blood was collected before morning feeding and at 0, 1, 6, and 12 h relative to bolus administration and nutrient restriction. Ewes were euthanized on d 17 for tissue collection. Plasma was analyzed for fatty acids (FA), glucose, and insulin using colorimetry and radioimmunoassay. Mass spec was used to quantify plasma Cer. As compared with CON, HIGH dose decreased metabolizable energy intake (−54%), BW and plasma insulin ($P < 0.05$). These effects were not observed for LOW or MOD. HIGH also increased plasma FA, relative to CON ($P < 0.05$). HIGH decreased plasma total, total dihydro- (DH), C20:0-, C22:0-, C24:0-, C26:0-, C22:0-DH, and C24:0-DH Cer concentrations by d 13 ($P < 0.05$), relative to CON. A similar but less-pronounced result was observed with MOD but not LOW. The ability of myriocin to acutely lower plasma Cer varied by species but was most pronounced 6–24 h post injection. During nutrient restriction, FA mobilization was lower within increasing dose ($P < 0.01$). Nutrient restriction affected plasma ceramides differently across time, based on dose and acyl moiety. Myriocin decreased the majority of Cer and DH-Cer measured in liver, omental and mesenteric adipose, and pancreas ($P < 0.05$). Myriocin only decreased C18:0-, C20:0-, and C18:0-DH Cer concentrations in skeletal muscle, and C18:0-Cer in subcutaneous adipose ($P < 0.05$). We conclude that the intravenous delivery of 0.3 mg of myriocin/kg of BW/48 h effectively decreases circulating and tissue ceramide concentrations without modifying energy intake in ruminants.

Key Words: ceramide, myriocin, ruminant

254 Hepatic gene expression of gluconeogenic enzymes and its regulation in Holstein cows under two contrasting feeding strategies. G. Cañibe*[1], M. Garcia-Roche[1,2], D. A. Mattiauda[1], A. Cassina[2], C. Quijano[2], and M. Carriquiry[1], [1]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República*, *Montevideo, Uruguay*, [2]*Centro de Investigaciones Biomédicas, Departamento de Bioquímica, Facultad de Medicina, Universidad de la República*, *Montevideo, Uruguay*.

The aim of the study was to determine the effect of pasture grazing on hepatic gene expression of key gluconeogenic enzymes and its regulation. Multiparous Holstein cows (n = 24, 664 ± 65 kgBW, 3.0 ± 0.4 BCS, spring calving) were assigned in a randomized block design to a total mixed ration (TMR) fed ad libitum (70% forage: 30% concentrate) (G0) or grazing plus supplementation (G1) from 0 to 180 d postpartum (DPP). The G1 cows grazed *Festuca arundinacea* or *Medicago sativa* in 2 (18 h) or one session (10 h) depending on heat stress (30 or 20 kgDM/d) and were supplemented with 5.4 kgDM/d of a commercial concentrate or offered TMR (50% of G0 offer). From 180 to 250 DPP, all cows grazed *Festuca arundinacea* (10 h; 30 kgDM/d) and were offered TMR (50% of G0 offer). Liver biopsies were collected at 35, 110 and 250 DPP. Expression of methylmalonyl-CoA mutase (*MCM*), pyruvate carboxylase (*PC*), phosphoenolpyruvate carboxykinase (*PCK*), glucose-6 phosphatase (*G6Pase*) and PPAG coactivator 1 α (*PGC1A*) mRNA was measured by real-time PCR, while AMPK phosphorylation was studied by western-blot. Data was analyzed with a mixed model using repeated measures. Hepatic *MCM* and *PC* mRNA was affected by the interaction between treatment and DPP ($P < 0.05$) as *MCM* was greater at 35 DPP but less at 110 DPP for G0 than G1 cows (0.93 vs. 0.56 ± 0.24, 0.61 vs. 0.89 ± 0.24, 0.59 vs. 0.65 ± 0.24, for 35, 110 and 250 DPP, G0 and G1, respectively); *PC* was 1.6-fold greater for G1 than G0 at 35 DPP. Expression of *G6Pase* and *PCK* mRNA was 1.6- and 1.9-fold greater ($P < 0.05$) while *PGC1A* mRNA tended ($P = 0.06$) to be 1.9-fold greater in early than mid and late lactation. The pAMPK/AMPK ratio was higher at 35 than 250 DPP (0.88 vs. 0.46 ± 0.08, $P < 0.01$). Hepatic *PGC1A* mRNA expression correlated positively with all gluconeogenic genes ($r > 0.3$, $P < 0.06$) while pAMPK/AMPK ratio correlated negatively with PC ($r = -0.4$, $P < 0.05$) suggesting both of these mechanisms could be regulating gluconeogenesis. Our results indicate cows under pasture-based systems adapt their metabolism to produce glucose from non-propionate when compared with TMR-fed cows.

Key Words: TMR vs. pasture, gluconeogenesis, 5’-AMP-activated protein kinase (AMPK)

255 Effects of dietary polyunsaturated fatty acid sources on lipid-related genes in bovine milk somatic cells. E. Vargas-Bello-Pérez*[1,2], N. Cancino-Padilla[1], C. Geldsetzer-Mendoza[1], M. S. Morales[3], H. Leskinen[4], P. C. Garnsworthy[5], J. J. Loor[6], and J. Romero[7], [1]*Departamento de*

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The objective of this study was to determine the effects of the number of double bonds of dietary lipids on expression of genes related to lipid metabolism in milk somatic cells (MSC) in dairy cows. For this, 15 dairy cows (2ndand 3th lactation, 42 L milk/day, 195 ± 35 d in milk) were randomly assigned to a control diet (CD) containing no added lipid (n = 5 cows); and diets supplemented with soybean oil (SO) (n = 5 cows; unrefined SO; 3% DM) or fish oil (FO) (n = 5 cows; manufactured from salmon oil; 3% DM); cows were fed for 63 d. On d 21, 42 and 63, MSC were obtained 4 h after first milking from all cows. Milk production, milk fat, and milk protein were not affected by treatments. Relative abundance of mRNA from 17 genes involved in lipid metabolic functions: fatty acid (FA) importation into cells, FA synthesis and desaturation, acetate and FA activation, FA intra-cellular transport, triacylglycerol synthesis, and lipid droplet formation regulation. Products of transcription (mRNA) from MSC were obtained by qPCR. The mRNA from cows fed CD on d 42 and 63 were compared with mRNA relative abundance at d 21 to evaluate fold-changes. Those genes from CD group without changes over the time (*ACACA*, *PPARGC1*, *LPIN1*, *INSIG1*, *DGAT1* and *FABP3*) were selected to analyze effects of SO and FO. The relative abundance software tool (REST) was used to analyze qPCR results. This software incorporates PCR efficiency correction and reference gene normalization. Compared with CD, SO downregulated ($P < 0.01$) *ACACA*, *INSIG1*, and *DGAT1*. Compared with CD, FO downregulated ($P < 0.01$) *ACACA*, *PPARGC1*, *LPIN1* and *FABP3*. Overall, data indicated that there are differential transcriptomic effects of lipid-related genes in MSC and that will depend on the number of double bonds of dietary lipids.

Key Words: oils, mammary gland, gene expression

256 Differential fates for gluconeogenic precursors in diverging Holstein genotypes.

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The aim of the study was to assess the effect of diverging Holstein genotypes in hepatic mitochondrial function and gene expression in a grazing system. Multiparous cows of New Zealand (NZH, n = 10) and North American origin (NAH, n = 10) (512 ± 19 vs. 563 ± 29 kg BW, 3.1 ± 0.1 body condition score (BCS), fall calving) were evaluated during the Spring of 3 consecutive years (2017, 2018 and 2019) to ensure maximum pasture allowance. Cows grazed a mixed pasture of *Medicago sativa* and *Dactylis glomerata* (15.4 ± 1.0 kg DM/d ha in 2 grazing sessions and were supplemented with 6.9 ± 0.5 DM/d of concentrate). Liver biopsies were collected 180 ± 17 d postpartum (DPP) and mitochondrial function was assessed measuring oxygen consumption rates using complex I (glutamate/malate: G/M) and complex II (succinate: succ) substrates; gene expression of pyruvate carboxylase (*PC*) and phosphoenolpyruvate carboxykinase (*PCK*) was measured by real-time PCR. Data were analyzed using a repeated model that included the genotype as the fixed effect and year and cow as random effects. In average, no differences were observed in solid-corrected milk yield (25.7 vs. 26.4 ± 1.2; $P = 0.38$) or BCS did not differ between genotypes NZH vs. NAH (2.6 ± 0.09, $P = 0.18$). The maximum respiratory capacity - related to the potential reserve capacity that could be used in cases of very high demands or insult - was higher for NZH than NAH (14.3 vs. 11.5 ± 1.9, for G/M and 50.2 vs. 26.7 ± 9.0 pmolO_2/min/mg for succ respectively, $P < 0.05$). Similarly, oligomycin-sensitive respiration - representing ATP-linked respiration - was higher for NZH than NAH (6.1 vs. 4.3 ± 1.9, for G/M and 9.3 vs. 5.2 ± 1.1 pmolO_2/min/mg for succ respectively, $P < 0.05$). Both parameters were 3-fold greater for succ than G/M-driven respiration. Both, hepatic *PC* and *PCK* mRNA were higher for NAH than NZH cows (1.4 vs. 0.6 ± 0.15 and 1.79 vs. 0.74 ± 0.24, $P < 0.05$). Negative correlations were found between succinate-driven respiratory parameters and expression of gluconeogenic genes ($r \geq -0.55$, $P < 0.05$). These results indicate that gluconeogenic precursors may have different metabolic fates: ATP synthesis vs. gluconeogenesis in NZH and NAH cows, respectively.

Key Words: grazing, mitochondria

419 Characterization of fatty acid esters of hydroxy fatty acids, a novel class of bioactive lipids, in milk fat of cows supplemented with stearic and palmitic acid.

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Fatty acid (FA) esters of hydroxy FA (FAHFA) are classified as estolides and have been characterized as potential anti-inflammatory and antidiabetic bioactive FA. FAHFA are classified into families according to the FA and hydroxy FA makeup [i.e., palmitic acid (PA) esters of hydroxystearic acid are PAHSA] and within each family multiple regioisomers exist depending on the location of the hydroxy group in the hydroxy FA (i.e., 9-PAHSA). The objective of this study was to characterize 5 FAHFA families made of PA, stearic (SA), palmitoleic (PO), or oleic (OA) acid (PAHSA, SAHSA, POHSA, PAHPA, OAHSA) in a retrospective analysis of milk fat samples from a fat supplement experiment. Briefly, 12 multiparous Holstein cows were arranged in a 4 × 4 Latin square. Treatments were a no supplement control (CON) or a 2% DM inclusion of either a highly enriched PA or SA or a combination of both (PA/SA). FAHFA families were detected by LC-TQMS using multiple reaction monitoring and quantified using a standard curve for each FAHFA regioisomer. Data were analyzed with a mixed model with cow and period as random effects and treatment as a fixed effect and a regression analysis was done between FAHFA and production traits and plasma metabolites. PA increased 9- and 12-PAHPA concentration 2.1- and 5.5-fold ($P \leq 0.01$ for both) and tended to decrease 10-PAHSA ($P = 0.07$), when compared with CON. There was no effect of treatment on 9-SAHSA, 9-OAHSA, 9-POHSA, 9-PAHSA, 12-PAHSA. Interestingly, the combination of PA/SA abolished the effect of PA on PAHPA levels. Notably, 12-PAHPA was positively related to milk 16 carbon FA ($R2 = 0.12$, $P = 0.02$) and plasma glucose ($R2 = 0.10$, $P = 0.04$) and negatively related to milk de novo FA ($R2 = 0.10$, $P = 0.03$). Additionally, 10-PAHSA was positively related to plasma nonesterified FA ($R2 = 0.26$, $P < 0.001$). The results, to the best of our knowledge, are the first characterization of FAHFA in bovine milk, suggest a role of dietary FA on FAHFA concentrations, and show that some FAHFA are correlated with production traits and plasma metabolites in Holstein dairy cows.

Key Words: fatty acid esters of hydroxy fatty acids (FAHFA), functional lipids, milk fat

Animal Behavior and Well-Being

257 Impact of pre- and postnatal heat stress on dairy calf behavior. B. Dado-Senn*, K. N. Gingerich, K. C. Horvath, S. L. Field, E. K. Miller-Cushon, and J. Laporta, *University of Florida*, *Gainesville, FL*.

Pre- and post-natal heat stress alters dairy calf thermoregulation and growth, but less is known about behavior. Herein, we characterized feed intake, activity patterns and cognitive function in dairy calves exposed to prenatal and/or postnatal heat stress. Holstein calves (n = 48) born to late-gestation heat-stressed or cooled dams (prenatal HT vs. CL; shade of barn vs. shade, fans, and soakers; ~46 d; THI >78) were exposed to heat stress or cooling postnatally (postnatal HT vs. CL; shade of barn vs. shade and fans; 56 d; THI >68), resulting in 4 treatments: HTHT, HTCL, CLHT, and CLCL; n = 12/treatment. At 2 d of age, calves were allotted 10 L/d of milk replacer (MR) ad libitum via automatic feeders (8 pens, n = 6 calves/pen where n = 3 calves/prenatal treatment/pen). A 1-d stepdown weaning started at 42 d. Intake was analyzed in daily intervals [0000–0700 (INT1), 0700–1300 (INT2), 1300–1900 (INT3), and 1900–2400 h (INT4)], and intake speed and feeder visits were averaged weekly. Calf activity was recorded (d2–56) via electronic leg-based accelerometers. At 35 d, a procedural learning task in a T-maze was used to assess initial and reversal learning (i.e., participation, number of sessions to pass task). Data were analyzed by PROC MIXED in SAS where calf was experimental unit. Postnatal HT calves consumed less MR relative to postnatal CL calves during INT2, and HTHT calves consumed less MR than CLCL calves during INT3 ($P < 0.01$). Prenatal HT drank MR slower relative to prenatal CL calves at 14 d ($P < 0.01$). Postnatal HT calves had fewer unrewarded visits ($P < 0.01$). Prenatal HT calves spent more time standing relative to prenatal CL calves (421 vs 389 ± 4 min; $P < 0.01$). There was a prenatal x postnatal interaction ($P \leq 0.05$) whereby CLHT calves had more standing bouts and decreased standing duration relative to other groups. More prenatal HT calves refused to drink MR in the T-maze task compared with prenatal CL calves (71 vs 100% participation; $P = 0.04$). Of calves that participated, the number of sessions to pass each learning stage did not differ ($P \geq 0.15$). Heat stress either pre- or postnatally impacts calf activity and feeding behavior with potential negative implications on welfare.

Key Words: activity, intake

258 A yearlong study: Effects of weather and animal characteristics on respiration rate in dairy cattle. G. Tresoldi*[1,2], M. Hejazi[1], and C. B. Tucker[2], [1]*College of Agriculture, California State University*, *Chico, CA*, [2]*Center for Animal Welfare, Department of Animal Science, University of California*, *Davis, CA*.

Respiration rate (RR) is often used to assess heat stress in cattle at earlier stages. However, it is unclear which RR thresholds indicate that cattle are hot. Throughout a year, we recorded weather variables and RR of 406 females (newborns to 6th lactation cows) totaling 11,210 records. Our goal was to determine which weather variable or thermal index best predicted RR and to evaluate how individual animal characteristics (e.g., animal type, breed, posture and location within pen) affected RR outcomes. Preliminary data analysis was done using mixed models, linear and logistic regression. In this study, RR ranged from 16 to 184 breaths/min. Air temperature (AT) alone best predicted RR among the 21 parameters tested. It accounted for 35% of the observed RR variation. During observations, AT ranged from 1.8 to 43.9°C. Our findings suggest that RR < 40 breaths/min, a threshold often considered the upper normal limit in veterinary textbooks, were unlikely. At 25°C, for example, only 1 in every 10 cows were estimated to have RR < 40 breaths/min. This AT is normally referred to as within the cow's thermoneutral zone. Overall, every 10°C increase in AT resulted in +14 breaths/min. Among animal type categories, calves were the most vulnerable, while dry cows were the least sensitive group (+17 and +11 breaths/min every 10°C, respectively, $P < 0.01$). Jersey animals were also more sensitive than Holsteins ($P = 0.02$), but the biological significance of the difference is unclear (+3 breaths/min every 10°C). RR were the lowest when lactating cows were near the feed bunk in comparison to other areas (46 vs. 53 breaths/min, respectively, $P < 0.01$). The latter can likely be attributed to the presence of soakers at the feedline. Finally, posture did not affect RR outcomes (lying vs. standing = 50 vs. 51 breaths/min; $P = 0.17$). In a Mediterranean climate, AT was the most reliable predictor of RR in dairy cattle. However, characteristics such as animal type, breed and location within pen should be taken into consideration when making decisions about heat load management. These decisions have important implications for the effectiveness of heat abatement strategies.

Key Words: heat stress, welfare, physiology

259 Effects of shade provision on the behavior of prepartum dairy cows in southern Chile. D. Cartes*[1], F. Matamala[1], A. Strappini[2], and P. Sepúlveda-Varas[3], [1]*Escuela de Graduados, Facultad de Ciencias Veterinarias, Universidad Austral de Chile*, *Valdivia, Chile*, [2]*Instituto de Ciencia Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile*, *Valdivia, Chile*, [3]*Instituto de Ciencias Clínicas Veterinarias, Facultad de Ciencias Veterinarias, Universidad Austral de Chile*, *Valdivia, Chile*.

The aim of this study was to investigate the effects of providing shade on the behavior of prepartum dairy cows managed outdoors and exposed to warm weather conditions in a temperate climate. Twenty-four multiparous Holstein prepartum dairy cows were randomly assigned to 6 groups approximately 21 d before their expected calving date based on BW, body condition score and parity. Each group (n = 4/group) were housed in open dirt corrals at the Experimental Dairy Farm of the Universidad Austral de Chile (Valdivia, Chile) during the summer months (January and February) until calving. Three groups had access to shade and 3 groups in adjacent corrals had no shade. Shade use and drinking time were daily recorded using scan sampling every 3 min during the hottest part of the day (1100 to 1800 h), whereas feeding time was daily recorded from 0830 to 2030 h. The daily lying time was measured with data loggers and rumination time was recorded using an automated monitoring system over the study period. Data were summarized by wk, compared between groups (shaded vs non-shaded) and analyzed using mixed linear models (R-Studio). On average, cows used shade during 47%, 46% and 20% of the observation period per day during wk 3, 2 and 1 before calving, respectively. During the morning, shaded cows spent more time feeding on wk 3 (72.6 vs. 59.4 min; $P = 0.02$), wk 2 (79.2 vs. 61.1 min; $P = 0.003$) and wk 1 (88.2 vs. 69.1 min; $P = 0.006$) before calving compared with non-shaded cows. Drinking time was lower in cows that had access to shade during the wk 3 (9.1 vs. 21.8 min; $P = 0.004$), wk 2 (11.6 vs. 20.4 min; $P = 0.05$) and wk 1 (11.0 vs. 20.8 min; $P = 0.02$) before calving compared with those without. Total time spent ruminating tended to be higher when cows had access to shade during the prepartum period compared with non-shaded cows (wk 3: 534 vs. 471 min/d; $P = 0.09$ / wk 2: 539 vs. 472 min/d; $P < 0.08$/ wk 1: 517 vs. 453 min/d; $P = 0.07$), but the daily lying time was not affected by shade treatment. These results confirm the importance of providing shade to prepartum dairy cows exposed to summer conditions under temperate regions. Project founded by FONDECYT 11170820

Key Words: shade, prepartum, dairy cow

260 Effect of episodic heat stress on behavior, body temperature, and lameness of lactating dairy cows on farms in northern New York. C. S. Ballard*, S. Green, S. Baldwin, A. E. Pape, and R. J. Grant, *William H. Miner Agricultural Research Institute*, *Chazy, NY*.

To assess the impact of episodic heat stress on lameness, resting time and body temperature of dairy cattle on farms in northern New York with varying degrees of heat abatement, a study was conducted from June through September 2019 on 4 Holstein herds. Farm A was a sand-bedded freestall with no mechanical ventilation in housing area. Farm B was a sand-bedded freestall with fans over stall beds. Farm C was a freestall, sawdust/mattress with fans/misters over feedbunk and stall beds. Farm D was a freestall, sawdust/mattress with fans over stall beds. Thirty cows with lameness score ≤2 served as a focal group averaging over 45.4 kg/d on each farm. Temperature and relative humidity were recorded continuously and temperature humidity index (THI) was calculated every 10 min. Wind speed (m/s) was measured weekly at standing and lying positions throughout pens. Lameness was scored at beginning and end of study. Lying behavior (h/d) and body temperature (reticular, °C) were measured continuously. Lameness (not lame vs. lame) from beginning to end of study was analyzed by Chi-squared analysis using Freq procedure (SAS 9.4). Retrospectively, 6 d of cool weather (mean THI <65, COOL) and 6 d of hot weather (mean THI ≥75, HOT) were selected and lying behavior and reticular temperature (RT, °C) data were analyzed by farm using Mixed procedure (SAS 9.4). All farms except Farm B increased lameness ($P < 0.05$) from beginning to end of summer. While all farms were impacted by heat stress ($P < 0.01$), Farm A was impacted the greatest with 21.4% decrease in lying time and 0.9°C increase in RT on HOT days. Farm B and C were impacted the least with 10.8% and 12.5% decrease in lying time and 0.4°C and 0.3°C increase in RT on HOT days, respectively. The wind speed on Farm A averaged 0.3 m/sec throughout the pen with least air flow in stall beds. Farms B and C had greater air flow in stall beds at resting level (1.0 and 0.9 m/sec, respectively) which likely resulted in less impact on lying time of cows during heat events compared with Farms A and D. Cows on all farms were impacted by episodic heat events to varying degrees with the cows without additional mechanical ventilation being impacted the greatest.

Key Words: heat stress, lying time, body temperature

Animal Health: Calves 3

261 The cost of bovine respiratory disease in Holstein replacement heifers. M. Overton*, *Elanco Animal Health*, *Greenfield, IN.*

Bovine respiratory disease (BRD) is a common disease concern in dairy cattle that is most commonly initially diagnosed in young dairy heifers. BRD has a negative impact at both the individual animal level and at the herd level, but this performance loss is highly variable, depending on disease severity, accuracy and completeness of clinical detection, effectiveness of treatment, and on-farm culling practices. Consequences include decreased rate of weight gain, a higher culling risk either as heifers or as cows, delayed age at first service, delayed age at first calving, and in some cases, lower future milk production. In this data set of 104,100 Holstein dairy replacement heifers from across the US, 36.6% had one or more cases diagnosed within the first 120 d of age with the highest risk of new cases occurring before weaning. Comparison of the raising cost for Holstein heifers with BRD and those without a recorded history of BRD resulted in an estimated cost per incident case occurring within the first 120 d of age of $252 or $282, depending upon whether anticipated future milk production differences were considered or not. No additional differences in first lactation performance such as culling risk or reproductive performance were evaluated. Current market conditions as reflected in this model have contributed to a cost estimate that is significantly higher than previously published estimates, driven in part by the losses associated with selective culling of a subset of heifers that experienced BRD. Depending on the available inventory of replacement heifers and the level of BRD within the herd, selective culling based on disease occurrence may limit a herd's ability to selectively cull based upon genetics; however, this financial cost was not considered here. The cost of BRD in dairy replacement heifers presented here is likely higher than many realize when all aspects of growth and performance are considered but may not completely capture the full economic losses.

Key Words: bovine respiratory disease, dairy replacement heifer, economics

262 Associations of serum protein concentrations with serum metabolites, average daily gain, and health measures during the early stages of growth in Holstein dairy calves. B. J. Tverdy*[1], C. Y. Tsai[1], H. C. Hung[1], P. Rezamand[1], and W. J. Price[2], [1]*Department of Animal and Veterinary Science, University of Idaho*, *Moscow, ID*, [2]*Statistical Programs, College of Agricultural and Life Sciences, University of Idaho*, *Moscow, ID.*

An objective of this study was to determine the associations of passive transfer status, by assessment of serum total protein (TP), with serum metabolites, daily gain, morbidity, and mortality in neonatal Holstein dairy calves (n = 1,558). Calves were purchased from dairy farms in the western United States and placed in a calf ranch as one day old. Calves were assigned an individual electronic identification and entered into Feedlot Health Management Services proprietary software, iFHMS (Feedlot Health Management Services, Preston, ID). Cause-specific morbidity and mortality was recorded for each calf daily from entry to exiting or death. A 5-mL tube of blood was collected from each animal at 48 ± 6h post-arrival. Whole blood was centrifuged at 2000 *g* for 10 min and serum was stored at −20°C. Serum TP was measured using a digital refractometer. Calves were categorized based on proposed AVMA serum TP guidelines into poor (TP <5.1 g/dL, n = 159, mean ± SD 4.68 ± 0.31 g/dL), fair (5.1 < TP ≤5.7 g/dL, n = 399, 5.45 ± 0.19 g/dL), good (5.8 ≤ TP ≤6.1 g/dL, n = 322, 5.96 ± 0.11 g/dL) and excellent (TP >6.1 g/dL, n = 751, 5.96 ± 0.11 g/dL). Samples were analyzed using a reverse-phase HPLC using a C18 column for vitamins and a colorimetric assay for glucose. Data were analyzed using GLIMMIX and logistic regression models with significance declared at $P \leq 0.05$. Significant differences between poor and excellent were observed in mortality and ear disease treatments ($P < 0.05$ for both). Differences were observed when comparing poor and fair to good and excellent TP categories for serum glucose ($P < 0.05$). Differences were also detected when comparing poor with all other groups for serum retinol ($P = 0.001$). Serum β-carotene and α-tocopherol were different when comparing all TP categories against the excellent ($P < 0.05$ for both). Average daily gain at 90 d and overall was not statistically different among TP categories. Overall, serum metabolites were different among TP categories, suggesting an associative relationship with health and the immune system.

Key Words: passive transfer, metabolites

263 Growth, rectal temperature, and health of male Holstein calves exposed to heat stress during pre-weaning. A. B. Montevecchio*[1], W. Frota[1], V. R. Merenda[1], J. G. Martin III[2], and R. C. Chebel[1], [1]*Department of Large Animal Clinical Sciences & Department of Animal Sciences, University of Florida*, *Gainesville, FL*, [2]*Dairy Design Engineers*, *Gainesville, FL.*

Objectives were to determine the effects of heat stress exposure of pre-weaned male Holstein calves on rectal temperature (RT), growth, and health. At birth, calves were paired according to the dam's parity (1st vs ≥2nd) and were assigned randomly to 1 of 3 treatments: 50% of the hutch covered with plywood and outside of the barn (HS = 20), hutch in a barn with no cooling (SH = 21), and hutch in a barn with cooling through forced air (SHF = 19). At birth, calves were weighed and, within 48 h, total protein in serum and failure of passive transfer (<5.5 vs. ≥ 5.5 g/dL) were determined. All calves were weighed (BW) and had the rump (RH) and wither (WH) heights measured at 12, 19, 26, 34, 47, 54, 61, and 71 d of age (weaning). Health was scored in the AM, including RT, every 3.5 ± 1.1 d from 2 to 68 d of age. A set of hutches (HS = 16, SH = 8, SHF = 15) was evaluated for air speed and temperature at 1000 and 1600 h and calves in these hutches had RT and respiratory frequency (RF) measured at the same time. Data were analyzed by ANOVA for repeated measures. At birth, treatments did not differ ($P > 0.10$) regarding BW, RH, WH, failure of passive transfer. The SHF treatment had ($P < 0.01$) the greatest air velocity (AM: HS = 0.60 ± 0.07, SH = 0.41 ± 0.09, SHF = 1.41 ± 0.09 m/sec; PM: HS = 0.66 ± 0.07, SH = 0.50 ± 0.10, SHF = 1.38 ± 0.10 m/sec) and HS treatment had ($P < 0.01$) the greatest air temperature (AM: HS = 33.40 ± 0.12, SH = 30.17 ± 0.19, SHF = 30.25 ± 0.16°C; PM: HS = 33.91 ± 0.18, SH = 32.74 ± 0.27, SHF = 32.61 ± 0.24°C). In the AM, SH calves had the greatest RT (HS = 38.66 ± 0.04, SH = 38.79 ± 0.04, SHF = 38.60 ± 0.04°C) but, in the PM, HS calves had the greatest RT (HS = 40.07 ± 0.07, SH = 39.05 ± 0.10, SHF = 38.97 ± 0.09°C). Calves in the HS treatment had the greatest RF (AM: HS = 73.63 ± 3.65, SH = 38.44 ± 3.05, SHF = 36.66 ± 2.58 mov/min; PM: HS = 92.27 ± 3.44, SH = 42.22 ± 2.56, SHF = 37.29 ± 1.95 mov/min). Treatment did not affect BW and average daily gain from 12 d of age to weaning, but HS calves had reduced RH (HS = 94.58 ± 0.70, SH = 96.29 ± 0.70, SHF = 97.10 ± 0.76 cm) and WH (HS = 88.52 ± 0.76, SH = 89.88 ± 0.76, SHF = 91.39 ± 0.83 cm) at weaning. Exposure to HS reduces body measurements of male Holstein calves.

Key Words: heat stress, Holstein, growth

264 Passive immunity and colostrum management practices on Ontario dairy farms and auction facilities: A cross-sectional study. C. B. Winder*[1], J. Marshall[1], B. Tuer[1], R. Genore[2], and D. L. Renaud[1,2], [1]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*, [2]*ACER Consulting*, *Guelph, ON, Canada.*

Failure of transfer of passive immunity (FTPI) in dairy calves has substantial health consequences, impacting well-being and performance. There have been no recent estimates of the prevalence of FTPI on Ontario dairy farms. The objectives of this cross-sectional study were to determine the level of FTPI in both male and female dairy calves across the province of Ontario, as well as determine colostrum management practices through an in-person questionnaire. One hundred and 9 dairy farms and 4 auction facilities in Ontario were visited in 2019. From the auction facilities, 386 male dairy calves were sampled and had a mean serum total protein (STP) of 5.79 g/dL (SD = 0.83). Using a cutpoint of 5.2 g/dL, 91 calves (24%) had FTPI. Of the 440 calves (201 male, 239 female) sampled on Ontario dairy farms between 24 h and 10 d of age, mean STP was 5.69 g/dL (SD = 0.78) and 107 calves (24%) had FPTI. The likelihood of FPTI was not associated with sex (female v. male, RR = 0.89, 95% CI = 0.64–1.24), age (per day, RR = 1.02, 95% CI = 0.94–1.11), or weight (per kg, RR = 0.98, 95% CI = 0.96–1.01). For first feeding of colostrum, median time to providing colostrum was 2.5 h (range = 0 to 12 h). Ninety-one farms (83%) fed colostrum from the dam of the calf as their predominant source of colostrum. The predominant feeding method for the first feeding of colostrum was a nipple bottle (89 farms, 82%). Twenty-seven farms (25%) reported managing colostrum differently for male calves, which included a different colostrum source (7 farms), use of poorer quality colostrum (3 farms), a smaller quantity of colostrum (3 farms), a longer time from birth to feeding (1 farm), and generally having a lower focus on colostrum management for male calves (9 farms). While the prevalence of FTPI on Ontario dairy farms appears to have improved since previous estimates, there remains substantial room for improvement. Although no overall differences were found in FTPI between male and female calves, differential reported colostrum management by sex indicates this may be a risk for male calves on a proportion of Ontario dairy farms.

Key Words: calf, failure of transfer of passive immunity (FTPI), failure of passive transfer (FPT)

265 *Lactobacillus animalis* LA51 and *Bacillus* sp. probiotics confer protection from the damaging effects of pathogenic *Clostridium perfringens* and *Escherichia coli* on the intestinal barrier.

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Toxins produced by *Clostridium* spp. can cause enteric disease in ruminant. The gut plays a key role in the digestion and absorption of nutrients and constitutes an initial organ exposed to external factors influencing the health of animals. Intestinal dysbiosis can promote overgrowth of different pathogens, which can cause intestinal barrier damage (leaky gut), which in turn may facility passage of toxins to the bloodstream. The objective of this study was to evaluate in vitro beneficial effects of different probiotic strains (*Lactobacillus animalis* (LA51), *Bacillus licheniformis* DSM5749 (BL) and *Bacillus subtilis* DSM5750 (BS)) on gut health in the presence of pathogens. Two assays were performed. For the adhesion assay, *E. coli* O157 (DSM17076) was added to intestinal Caco-2 cell monolayers (3×10^7 cfu/well) pre-incubated or not with BL or BS (1.5×10^9 cfu/well). *E. coli* adhesion was quantified by cfu enumeration using MacConkey agar plates incubated 18h at 37°C. For the “leaky gut” assay, transepithelial electrical resistance (TEER) was measured across Caco-2 monolayers exposed to live or dead LA51 ($2\text{x}10^8$ cfu/transwell) with or without *Clostridium perfringens* type a (CPa) (DSM756, $2\text{x}10^7$cfu/transwell). FITC-dextran (FD) was added to the apical side of the Caco-2 cells after 5h of TEER measurements. The amount of FD translocated to the basolateral side was quantified after 5h by measuring the fluorescent signal. BL and BS reduced the binding of *E. coli* O157 to the cells by 78% and 51%, respectively (6.7×10^6 cfu/mL vs. 1.5×10^6 or 3.3×10^6 cfu/mL, $P < 0.01$). CPa caused a TEER decrease over the time, while live (but not dead) LA51 significantly reduced the TEER decrease (15 Ω cm^2 vs. 150 Ω cm^2, $P < 0.01$) and the amount of FD translocation (5.7% vs. 0.1%, $P < 0.01$). In conclusion, LA51 confers protection against *Clostridium perfringens type a* by counteracting its damaging effect on the intestinal integrity, while BL and BS reduce the adherence in vitro of pathogenic *E. coli*.

Key Words: *Lactobacillus animalis*, *Clostridium perfringens*, *Bacillus licheniformis*

266 Effects of feeding *Saccharomyces cerevisiae* fermentation products on the health of Holstein dairy calves following a lipopolysaccharide (LPS) challenge.

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Our objective was to evaluate the effect of *Saccharomyces cerevisiae* fermentation products (SCFP) on the immune status of calves, just before weaning, following an LPS challenge. Calves were blocked by BW and serum total protein and assigned to 1 of 2 treatments; CON (24% CP:17% fat milk replacer (MR) and an 18% CP starter) or SCFP (24% CP:17% fat MR with 1 g/d of SmartCare (Diamond V) and an 18% CP starter with 0.8% NutriTek (Diamond V)). Calves were offered 2.84 L (12.5% solids) of MR twice daily (0630 and 1630 h). Calves received ad libitum access to a texturized starter and water. On d 49 (pre-challenge) and d 52 (post-challenge), blood was collected to measure blood fractions (Genesis, Oxford Science, Oxford, CT). On d 50, 20 calves (10/treatment) were enrolled in an LPS (*E. coli* O111:B4) challenge. Calves were intravenously dosed with 0.125 µg/kg of BW. At −1.5, −0.5, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, and 24 h, relative to LPS, 20 mL of blood was collected via a jugular catheter, and temperature and respiratory rate were measured. Data were analyzed as a completely randomized block design in SAS v.9.4 with repeated measures. At 0.5 h, SCFP calves had an increased temperature (39.5°C vs. 39.0°C; $P = 0.04$) and respiration rate (103 vs. 80 breaths/min; $P = 0.002$) compared with CON calves. At 1.5 h, SCFP calves had an increased respiration rate (82 vs. 64 breaths/min; $P = 0.02$) compared with CON calves. On the day of the LPS challenge, SCFP calves consumed 933 g less starter compared with the CON calves ($P = 0.002$). There were no intake differences after the day of the LPS challenge ($P > 0.29$). For the blood fractions, there was a significant time point effect for white blood cells (10^3/µL), lymphocytes (10^3/µL), eosinophils (10^3/µL and %), basophils (10^3/µL and %), red blood cells (10^6/µL), and hemoglobin (g/dL; all $P < 0.01$). This suggests an LPS challenge impacted blood fractions of calves, indicating immune system activation. Using an LPS model, SCFP caused a more acute response, potentially because of a primed immune system.

Key Words: calf, *Saccharomyces cerevisiae* fermentation products, LPS challenge

267 Rumination time in early lactation is associated with peak milk yield. M. Peiter*, H. N. Phillips, and M. I. Endres, *Department of Animal Science, University of Minnesota*, *Saint Paul, MN.*

Automatic milking systems (AMS) may include individual cow sensors that measure daily rumination time (RT) and activity. Total daily RT is normally reduced during the peripartum period and stabilizes to normal after the first week of lactation. Rumination time has been shown to be associated with health and milk yield. Peak milk yield (PMY) has a positive relationship with overall 305-d milk yield. Therefore, the objective of this study was to investigate the association between change in RT and average daily RT during the first 7 DIM and PMY. Daily cow data were collected from 34 free-flow AMS farms (n = 3,227 cows) in MN and WI. A simple linear regression line of best fit was estimated for RT over the first 7 DIM for each cow to calculate the change in RT (slope coefficient). Two separate linear mixed models were used with PMY as the outcome variable. Change in RT, parity (1, 2, and 3+), and the interaction term were included as fixed effects in the first model (M1). The other model (M2) used average RT during the first 7 DIM, parity, and the interaction term as fixed effects. Farm and cow within farm were included as random effects in both models. Statistical analyses were performed using R. Least squares means (±SE) for PMY were 42.3 ± 0.78, 56.3 ± 0.76, and 60.3 ± 0.76, and average RT was 365, 401, and 393 min/d for parity 1, 2, and 3+, respectively. For M1 no interactions were found. For each 10-unit increase in change in RT, PMY increased by (coefficient [95% CI]) 5.9 [2.0, 9.8], 10.3 [7.5, 13.1], and 11.9 [9.0, 14.7] kg for parity 1, 2, and 3+, respectively. In M2, for each 10-unit increase in average RT, PMY increased by 1.3 [0.5, 2.0] and 1.4 [0.6, 2.2] kg for parity 2 and 3+, respectively; however, average RT was not associated with PMY for parity 1 (0.2 [-0.9, 1.3] kg). Change in RT was a better predictor of PMY compared with average RT for all parity groups, and especially for cows of parity ≥2. Results show that cows that increase RT at a faster rate after calving will peak higher, which most likely lead to higher overall milk yield.

Key Words: rumination, peak milk yield, automatic milking system

268 The effects of morning compared with afternoon feed delivery on milk production, feed intake, and feeding behavior in lactating dairy cows. P. D. French*[1] and C. S. Shugart[2], [1]*PHD R&D LLC*, *Fort Atkinson, WI*, [2]*Iowa State University*, *Ames, IA.*

Delivering fresh feed once daily in the PM versus the AM may improve feed efficiency but alters feeding behavior of cows housed in tie-stalls. We evaluated effects of once daily TMR delivery on production and efficiency of group-housed, individually fed dairy cows. Twelve, multiparous Holstein cows (110 ± 13 DIM) with a preliminary milk yield of 52 ± 10 kg/d were used in a crossover design with 14-d periods. The last week of each period was used for data collection. Electronic feeding stations (BioControl CRFI, Rakkestad, Norway) continuously recorded the timing of feed intake. Treatments were fed ad libitum with feed delivery 1x/d at 0830 or 1630 h. The TMR contained 54% forage, 16.4% CP, 28.2% NDF, and 27.4% starch. Cows were milked 3x/d and housed in a pen with a single row of freestalls. Data were analyzed using the mixed model procedure of SAS. Average temperature during period 1 and 2 was 23°C and 25°C, respectively. DMI was greater for PM fed cows (32.2 vs 29.4 kg/d, $P < 0.01$). Milk yield (52.6 kg/d), milk composition (3.6% fat and 3.0% protein), and milk component yield (1.87 kg/d fat and 1.56 kg/d protein) did not differ for time of feeding. Body weight was greater (734 vs 727 kg, $P < 0.05$) and BW gain tended to be greater for PM fed cows. Meals per day (9.7 vs 8.6, $P < 0.05$) and eating rate (159 vs 149 g DM/min, $P < 0.05$) were greater for PM fed cows. Daily eating time (209 vs 201 min/d, $P < 0.07$) tended to be greater for PM fed cows. DMI per meal, meal size, and rumination time were similar for AM and PM feeding. In the 6 h following TMR delivery, PM fed cows consumed more DM (14.2 vs 10.8 kg, $P < 0.01$) and spent more time eating (86 vs 66 min, $P < 0.01$). In the interval between 0830 and 1430 h, AM cows consumed more DM (10.7 vs 4.5 kg, $P < 0.01$), whereas PM fed cows consumed more DM from 1430 to 2030h (13.1 vs 10.2, $P < 0.01$). More importantly, PM cows consumed more DM during the 0230–0830h interval (6.5 vs 2.9 kg, $P < 0.01$). In conclusion, offering TMR in the PM compared with AM altered feeding behavior that led to lower feed efficiency.

Key Words: feeding behavior, feed efficiency

269 Increase of feed efficiency in early lactation dairy cows using butafosfan associated or not with cyanocobalamin. C. Pizoni*, J. Halfen, V. S. Izquierdo, R. Klaus, E. dos Santos, L. V. Vieira, E. Schmitt, A. A. Barbosa, K. B. Cardoso, B. E. Velasquez, C. C. Brauner, J. O. Feijó, V. R. Rabassa, F. A. B. Del Pino, M. N. Corrêa, *Núcleo de Pesquisa, Ensino e Extensão em Pecuaria (NUPEEC), Departamento de Clínicas Veterinaria, Universidade Federal de Pelotas*, *Pelotas, Rio Grande do Sul, Brazil.*

The butafosfan with or without cyanocobalamin have been used in early postpartum dairy cows for many years as a strategy to improve the energetic status in cow. The study aimed to evaluate the effect of butafosfan combined or not whit cyanocobalamin on dry matter intake, milk production and metabolism of early lactation dairy cows. Thirty multiparous Holstein cows managed in a compost barn system were enrolled in the trial at the calving date and remained on experiment until 28 DIM. Cows were assigned to 1 of 3 groups: Butafosfan (5mL/100kg of BW, n = 10); Catosal (25mL/animal butafosfan+cyanocobalamin, n = 10); and Control group (25mL/animal, n = 10, NaCl 0.9%) injections at calving (0 d), 3 and 7 DIM. The DMI was evaluated until 21 d and milk production until 28 d of experiment. Blood samples were collected at 0, 3, 7, 14, 21 and 28 d to analyzed NEFA, BHB, acetone and glucose. The data were analyzed using the MIXED procedure of SAS. The DMI was lower in Butafosfan ($P < 0.05$) when compared with Catosal and Control groups (17.470 ± 0.93 kg vs. 20.58 ± 0,93 kg vs. 21.10 ± 0.77 kg of DMI), which resulted in an average decrease of 3.4 kg. Milk production was greater ($P < 0.05$) in Catosal when compared with Control, which resulted in an increase of 7.8 kg/milk/d, however Butafosfan group was similar ($P > 0.05$) than the others (42.17 ± 2.47 vs 34.79 ± 1.73 vs 37.78 ± 1.56), respectively. The feed efficiency was greater ($P < 0.01$) in Catosal group in compare with the others, while Butafosfan was greater than Control (2.26 ± 0.10 vs. 1.87 ± 0.10 vs. 1.53 ± 0.08), respectively, which can be associated with greater milk production and maintenance of DMI in this group. No treatment effect ($P > 0.05$), was observed for serum glucose, NEFA and BHB concentrations, however, acetone showed the lowest concentration ($P < 0.05$) in Catosal group than Butafosfan and Control (1.02 ± 0.03 vs. 1.40 ± 0.03 vs. 1.42 ± 0.04). No effect was observed for milk composition ($P > 0.05$). These results suggest that the injections of Butaphosphan and Catosal may improve milk production and feed efficiency by modulating DMI.

Key Words: organic phosphorus, fresh cows, dry matter intake

270 The effect of metaphylactic use of tildipirosin for the control of respiratory disease associated with long-distance transportation of dairy calves. M. Celestino*[1], L. Fernandes[1], P. Menta[1], D. Paiva[1], T. Ribeiro[1], T. Silva[1,2], R. Neves[1,3], M. Ballou[1], and V. Machado[1], [1]*Texas Tech University*, *Lubbock, TX*, [2]*University of São Paulo*, *Pirassununga, São Paulo, Brazil*, [3]*Purdue University*, *West Lafayette, IN.*

The objective of this study was to evaluate the efficacy of 2 metaphylactic strategies using tildipirosin in controlling bovine respiratory disease (BRD) incidence for dairy calves that were transported within their 1st wk of life. A total of 2,100 calves were randomly allocated into 3 groups at 3 d after arrival: META1 calves received a single subcutaneous (SQ) injection of tildipirosin (Zuprevo, Merck Animal Health) at enrollment;

META2 calves received one SQ injection of tildipirosin at enrollment and 17 d later; and CON calves remained as untreated controls. A validated scoring chart (UC Davis) was used for BRD diagnosis. Ultrasonography (US) of the lungs was assessed for a random subset of 200 calves per treatment-group, and blood collected for the measurement of stress and inflammatory markers. Data were analyzed using multivariate logistic regression, Cox Proportional Hazards models, repeated measures ANOVA, and multivariate linear regression models. Metaphylactic use of tildipirosin did not affect BRD incidence ($P = 0.44$), presence of lung lesions diagnosed by US at weaning ($P = 0.99$) and average daily gain ($P = 0.25$). There was a tendency for META2 to decrease mortality in comparison to CON calves ($P = 0.05$). At 27 d after enrollment, calves enrolled in META 2 had decreased concentrations of haptoglobin and aspartate aminotransferase, and decreased neutrophil to lymphocyte ratio compared with CON calves ($P < 0.05$). Additionally, CON calves had increased concentration of globulins and lower albumin to globulin ratio than META2 calves at the end of the weaning period ($P < 0.05$). Metaphylactic use of tildipirosin in calves transported within the first week of life did not reduce BRD incidence before weaning despite reducing concentrations of inflammatory markers and enhancing immune parameters. Metaphylaxis tended to decrease mortality during the pre-weaning period.

Key Words: calves, metaphylaxis, bovine respiratory disease (BRD)

271 Impact of heat stress and OmniGen-AF on performance and immunity of mid-lactation dairy cows. T. N. Marins*[1], J. Gao[1], Q. Yang[1], R. M. Binda[1], C. M. B. Pessoa[1], R. M. Orellana[1], J. K. Bernard[1], M. Garcia[2], D. J. McLean[2], J. D. Chapman[2], D. J. Kirk[2], and S. Tao[1], [1]*University of Georgia*, *Tifton, GA*, [2]*Phibro Animal Health Corp*, *Teaneck, NJ.*

This study aimed to evaluate the impact of OmniGen-AF (OG; Phibro Animal Health) supplementation and deprivation of evaporative cooling on performance and peripheral blood mononuclear cells (PBMC) proliferation of mid-lactation Holstein cows during summer. Multiparous cows (n = 60) were blocked by parity (2.5) and DIM (189 d), and randomly assigned to 4 treatments (TRT) in a 2 × 2 factorial arrangement with 2 environments (E): cooled (CL) using fans and misters or non-cooled (NC), and 2 top-dressed feed additives (D, 56 g/d): OG or AB20 (Phibro Animal Health) as control (CON). Cows were cooled and fed respective diets for 60 d before experiment. Temperature-humidity index averaged 78 during the 8 wk trial. Dry matter intake (DMI) was recorded daily and milk yield (MY) 3x/d. Vaginal temperatures (VT) were recorded every 5 min for 4 d/wk. Blood was drawn on d 1, 3, 5, 7, 15, 28, 44, and 56 to analyze cortisol (COR), prolactin (PRL), tumor necrosis factor (TNF)-α and interleukin (IL) 10. PBMC were isolated on d 3, 14, and 42 (n = 8 cows/TRT) and cultured with hydrocortisone, PRL, lipopolysaccharides (LPS), or their combinations to assess proliferation and cytokine production. NC cows had higher VT, lower DMI and MY (E: $P < 0.01$), and greater blood COR at d 1 and 5 than CL (E × Time: $P < 0.01$). NC did not alter COR levels in OG cows but NC-CON cows had higher COR than CL-CON (E × D: $P = 0.02$). NC did not alter TNF-α levels in OG cows but NC-CON cows tended to have lower TNF-α than CL-CON (E × D, $P = 0.07$). LPS+hydrocortisone-stimulated PBMC from OG cows tended ($P = 0.09$) to proliferate more than CON. Deprivation of cooling tended to increased proliferation of LPS- and LPS+PRL-stimulated PBMC from OG cows, but not CON cows (E × D: $P \leq 0.108$). Relative to CL, PBMC supernatant of NC cows had greater ($P \leq 0.01$) TNF-α concentration stimulated by LPS or LPS+PRL, and tended ($P = 0.09$) to have greater IL10 stimulated by LPS, and those were not affected by diet. In conclusion, NC impaired performance and promoted COR release, but OG supplementation prevented COR release, maintained TNF-α secretion and promoted PBMC proliferation after deprivation of cooling.

Key Words: heat stress, OmniGen, immunity

Reproduction

272 Effects of feeding rumen-protected lysine during the transition period on postpartum uterine health and follicular dynamics of Holstein cows.

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Diets fed during the transition period play an important role in the cow's reproductive tract following parturition. We aimed to determine the effects of feeding rumen-protected Lys (RPL, AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) pre- (0.54%DM of TMR) and/or postpartum (0.395%DM of TMR) on uterine health and follicular dynamics of Holstein cows ($n = 75$). Treatments began at 28d before expected calving and consisted of TMR with RPL pre- and postpartum (LL), with RPL pre- and without RPL postpartum (LC), without RPL pre- and with RPL postpartum (CL), and without RPL pre- and postpartum (CC). Uterine health was evaluated at 4, 7, 10, 13, 15, and 17 DRC by evaluating purulent vaginal discharge (PVD) via Metricheck (MC) Score and MC Smell. Swabs of the endometrium were collected at 15 and 28 DRC. Polymorphonuclear leukocytes (PMN) were evaluated as a percentage of the epithelial cells. Follicular development was monitored via ultrasound every 2 d starting at 7 DRC until 28 DRC. Data were analyzed using MIXED and GLIMMIX procedures in SAS. Cows in C (20.39 ± 3.49%) had greater ($P < 0.01$) proportion of PMN cells in the uterus than cows in L (14.02 ± 3.49%); and there was also an effect of POS on PMN, in which cows in LC (18.88 ± 3.49%) had greater ($P < 0.01$) postpartum proportion of PMN cells in the uterus than cows in LL (9.16 ± 3.49%). A PRE × POS interaction for PMN was present, as cows in LL (9.16 ± 3.49%) had lower ($P < 0.01$) PMN proportion than cows in LC (18.88 ± 3.49%), CL (20.50 ± 3.49%), and CC (20.76 ± 3.49%). Cows in CL were more likely to develop PVD than cows in LL (95CI = 2.08–61.91, $P = 0.01$) or LC (95CI = 1.33–23.55, $P = 0.02$) at 13 DRC. A POS effect ($P = 0.02$) on the total growth of the first dominant follicle (DF) was detected, in which cows in LC (11.19 ± 1.68mm) and CC (11.34 ± 1.68mm) had greater total growth of DF than cows in LL (6.32 ± 1.68mm) and CL (7.73 ± 1.68mm). In conclusion, feeding cows with RPL pre- and postpartum improved uterine health postpartum, and the inclusion of RPL postpartum decreased growth of the first DF.

Key Words: lysine, polymorphonuclear leukocytes, purulent vaginal discharge

273 Does timing of AI affect P/AI in seasonal-calving, pasture-based lactating dairy cows inseminated with sex-sorted sperm?

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The objective was to use ovulation synchronization with timed artificial insemination (TAI) to evaluate the effect of timing of AI (16 h or 22 h after second GnRH) with frozen sex-sorted sperm on fertility performance in pasture-based dairy cows. Ejaculates from three Holstein-Friesian bulls were split and processed to provide frozen sex-sorted sperm at 4×10^6 sperm per straw (SexedUltra-4M; SS) and frozen conventional sperm at 15×10^6 sperm per straw (CONV). A modified Ovsynch protocol with a PRID inserted for 8 days was used to synchronize ovulation in 2175 cows on 24 herds. Cows were randomly assigned to receive TAI 16 h after the second GnRH injection for cows assigned to CONV and either 16 h (SS-16) or 22 h (SS-22) for cows assigned to SS ($n = 725$ cows/treatment). All cows were only used for first AI. Generalized linear mixed models were used to examine effects on pregnancy per artificial insemination (P/AI). P/AI was greater (P<0.001) for CONV compared to both SS-16 and SS-22 (61.0%, 49.3% and 51.3%, respectively), and the SS treatments did not differ from each other (relative P/AI for SS-16 and SS-22 vs. CONV were 80.8% and 83.9%, respectively). There were significant bull and treatment by bull interaction effects. Marked herd to herd variation in relative P/AI was observed (range: 50.9% to 116.8%). The best third of herds achieved a mean relative P/AI of 101.5% (range = 93.9% to 116.8%), indicating that P/AI equivalent to CONV was achieved with SS. Conversely, the lower third of herds achieved a mean relative P/AI of 67.4% (range = 50.9% to 76.9%). All herds had acceptable P/AI with CONV (range 53.6% to 71.0%), indicating good response to the synchronization protocol and induction of a fertile ovulation event. Identification of factors responsible for the large herd to herd variation in P/AI with SS, and development of protocols to reduce this variation, warrant further research.

Key Words: sex-sorted sperm, synchronization, fixed time AI

274 Effect of three different schemes of ovum pick-up on the follicular population, recovery, quality, and in vitro developmental competence of oocytes in Sahiwal cattle.

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The objective of the present study was to compare the 3 different schemes of ovum pick-up (OPU) in terms of follicular population, recovery, quality and nuclear maturation of oocytes, and in vitro developmental competence of embryos in Sahiwal cattle. Eighteen ($n = 18$) Sahiwal cattle (having a normal estrous cycle) were selected and subjected to a 7-d double-dominant follicle puncture (DFP). All the animals were randomly divided into 3 groups following DFP: (1) Twice-weekly ($n = 6$), (2) Once-weekly ($n = 6$), and (3) Bi-weekly ($n = 6$) OPU interval groups. The first OPU was conducted on either 3 to 4 d, 7 d or 14 d after 2nd DFP, depending on the treatment recommended for each group and a total of 60 ($n = 60$) OPU's were performed for each group. Data on the follicular population, oocytes recovery and total viable oocytes were analyzed by One-way ANOVA and LSD test was used to test the difference between the groups. The data on the oocytes quality, nuclear maturation, cleavage rate and blastocyst rate were analyzed by chi-squared test using SPSS. Results (Mean ± SE) and (%) showed that the number of medium-sized follicles (1.16 ± 0.21 vs. 0.86 ± 0.12 vs. 0.55 ± 0.09) and A, B grade oocytes (33.4 vs. 21.5 vs. 21.8%) in the twice-weekly OPU interval group were higher ($P < 0.05$) compared with once-weekly and bi-weekly OPU interval groups, respectively. The number of oocytes recovered (6.80 ± 0.96 *vs*. 4.80 ± 0.42 *vs*. 4.20 ± 0.40) and degenerated oocytes (0.85 ± 0.20 *vs*. 0.43 ± 0.12 *vs*. 0.16 ± 0.06) were higher ($P < 0.05$) in bi-weekly OPU interval group compared with once-weekly and twice-weekly OPU interval group, respectively. Nevertheless, total viable oocytes (2.70 ± 0.28 vs. 2.60 ± 0.27 vs. 3.30 ± 0.46), nuclear maturation (63.6 vs. 51.4 vs. 59.2%), cleavage rate (59.3 vs. 44.2 vs. 56.6) and blastocyst rate (29.0 vs. 18.5 vs. 28.3%) did not differ ($P > 0.05$) between the groups. In conclusion, twice-weekly OPU interval is a good approach for maximizing Sahiwal cattle's genetic potential within a short period of time without sacrificing the total transferable stage embryos.

Key Words: ovum pick-up, Sahiwal cattle

275 Association between delayed clinical cure and culling in dairy cows diagnosed with metritis.

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Objectives were to assess the risk and timing of culling associated with delayed clinical cure in dairy cows diagnosed with metritis. This prospective cohort study compiled data from 4 experiments performed between 2012 and 2018 in 6 herds located in Florida. Metricheck device was used to evaluate vaginal discharge (VD) within 21 DIM (1 = clear mucus; 2 = mucus with few flecks of pus; 3, = mucus with <50% of pus; 4, = mucus with ≥50% of pus; 5 = watery, fetid, reddish-brownish). Cows with VD = 5 were diagnosed with metritis. Cows diagnosed with metritis (d 0) were treated with either ampicillin or 2 formulations of ceftiofur and paired with cows without metritis counterparts (NoMet; n = 2,906) based on calving date and parity. Cows previously diagnosed with metritis that displayed VD <5 on d 11, were classified as cured (MetC; n = 1,136) and those with VD = 5 were classified as not cured (MetN; n = 279). Risk of culling within 300 DIM was analyzed by logistic regression. Hazard of culling within 60 and 300 DIM were evaluated by Cox's proportional hazard models. Tukey method was used to correct for multiple comparison. Risk of culling was greater ($P < 0.02$) for MetN compared with NoMet or MetC (22.4, 14.3, and 15.6%). Hazard of culling within 60 DIM was greater ($P \leq 0.001$) for MetN compared with MetC (AHR = 2.25; 95% CI = 2.09 to 2.41) or NoMet (AHR = 2.73; 95% CI = 1.75 to 4.25), whereas no difference was observed ($P = 0.30$) between MetC and NoMet (AHR = 1.21; 95% CI = 0.84 to 1.75). Average intervals to culling were 54 DIM for MetN, 58 DIM for MetC, and 58 DIM for NoMet. Conversely, no difference ($P = 0.68$) was observed for the hazard of culling within 300 DIM, and the AHR for MetN compared with MetC was 1.15 (95% CI = 0.89 to 1.41), and NoMet was 1.07 (95% CI = 0.75 to 1.51). No difference ($P = 0.72$) was observed for MetC compared with NoMet (AHR = 0.93; 95% CI = 0.73 to 1.18). Average days to culling were 283 DIM for MetN, 287 DIM for MetC, and 300 DIM for NoMet. Delayed clinical cure of metritis was associated with increased hazard of culling and earlier exit from the herd within the first 2 mo postpartum.

Key Words: uterine disease, health, culling

276 An electronically controlled intravaginal hormone delivery device successfully induced luteal regression in dairy cattle.

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Our objective was to develop and validate an electronically-controlled hormone delivery device for reproductive control of cattle. After development and in vitro testing of a prototype device for intravaginal (IVG) hormone release, we aimed to demonstrate the feasibility of inducing luteal regression by automated treatment with PGF_{2a} (PGF). The IVG device comprises an outer 3D-printed plastic housing, fluid reservoirs connected to delivery pumps and tubing, a programmable circuit board, and a retention mechanism. For in vitro testing, 4 pumps were programmed to release different target volumes (0.1, 0.2, 0.5, 1.0, and 2.0 g) in 4 replicates (n = 80). A Bland-Altman plot was constructed to assess the magnitude of disagreement between expected and delivered volumes. Observations fell within acceptable limits of agreement (1.96 SD) > 95% of the time, indicating overall good agreement (mean difference = 0.008 g). To assess in vivo performance of the IVG device, lactating Holstein cows with at least 1 corpus luteum = 15 mm in diameter were randomly allocated to 1 of 3 treatments: IM-PGF (n = 6); 2 25 mg intramuscular doses of PGF (Dinoprost) 24 h apart, DEV-PGF (n = 6); 4 25 mg doses of PGF released automatically by the IVG device every 12 h, and DEV-CTL (n = 4); insertion of an empty IVG device (placebo control). Blood samples were collected at 0, 12, 24, 36, 48, and 72 h after treatment. Data were analyzed by ANOVA with repeated measures in SAS. All devices (10/10) remained in situ until removed at 48 h. Progesterone (P4) concentrations from 0 to 72 h were affected by treatment ($P < 0.01$), time ($P < 0.01$), and their interaction ($P < 0.01$). Concentrations of P4 did not differ at time 0 but differed from 24 to 72 h as cows in IM-PGF and DEV-PGF had lesser P4 than cows in DEV-CTL. Conversely, P4 did not differ for IM-PGF and DEV-PGF during the experiment. We conclude that the current IVG hormone releasing device prototype can be programmed to automatically release PGF for successful induction of luteal regression in lactating dairy cows.

Key Words: automation, hormone delivery, dairy cow

277 Associations between pregnancy associated glycoproteins in milk and calving characteristics in seasonal-calving pasture-based dairy cows.

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The objective was to retrospectively examine the effect of calf sex (CS; female, male), calf birth weight (CBW; small ≤ 30kg, medium 30–36kg, large ≥ 36kg) and calving difficulty (CD; normal or difficult) on weekly milk PAG S-N values between wk 5 and 21 of pregnancy. Only cows that conceived to first AI and maintained pregnancy were analyzed (n = 259). PAG S-N values were not normally distributed. An appropriate Box-Cox transformation was identified, therefore, and the transformed PAG S-N values were analyzed using generalized linear mixed-models (presented as back-transformed LS Means and 95% CI). There was an interaction ($P = 0.001$) between CS and week because pregnancies with female offspring had greater PAG S-N at wk 5 (2.11, [1.92, 2.30]) and 6 (1.88, [1.77, 1.99]) of pregnancy compared with pregnancies with male offspring (1.98, [1.81, 2.15] and 1.77 [1.66, 1.89]). An interaction ($P < 0.001$) between CBW and week of pregnancy was detected whereby medium CBW calves had greater PAG S-N values at wk 5 (2.20 [1.96, 2.45]) and 6 (1.95, [1.79, 2.1]) compared with low (1.83 [1.62, 2.05] and 1.74 [1.58, 1.90]) and high (2.10 [1.90, 2.32] and 1.80 [1.66, 1.95]) CBW calves, respectively. Pregnancies with female offspring had greater ($P < 0.001$) PAG S-N values pre-nadir (1.83, [1.80, 1.86]) and lesser values post nadir (2.50, [2.48, 2.52]) versus pregnancies with male offspring (1.67 [1.64, 1.70] and 2.56 [2.53, 2.58], respectively). Pregnancies with low CBW calves had greater ($P < 0.001$) pre-nadir peak S-N values (1.84 [1.81, 1.87]) than pregnancies with medium (1.73 [1.69, 1.76]) and large (1.69 [1.65, 1.71]) CBW calves. Normal CD had greater ($P = 0.01$) pre nadir PAG S-N values (1.78, [1.76, 1.80]) compared with cows with a difficult CD (1.72, [1.68, 1.76]). Cows carrying a female calf or carrying calves with less CBW had the greatest milk PAG S-N. Calf characteristics were associated with differences in weekly PAG S-N values. Calf characteristics, including CS and CBW, were associated with prior differences in weekly milk PAG S-N values between wk 5 and 21 of pregnancy.

Key Words: embryo, calf, pasture

278 Dietary energy source effects on pregnancy rates and progesterone concentrations in heifers.

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Increasing dietary glucogenic precursors may improve progesterone (P4) production and conception rates in heifers. This study measured the effects of isoenergetic, isonitrogenous diets differing in primary energy source (starch or fat) on pregnancy rates and P4 concentrations of pastured heifers. Angus cross heifers (n = 29) were balanced on BW and condition score and assigned to 1 of 2 dietary treatments: a feed supplement high in starch (27.48% starch, 2.73% fat) (n = 15) or high in fat (n = 14, 9.13% starch 9.13% fat) (n = 14). Animals were housed in 1 of 2 pastures from March to July of 2019. Treatments were delivered using the SmartFeed individualized feeding system, allowing up to 4.54 kg/d of the supplemental feed. Feed delivery began 18 d before breeding and continued through the second pregnancy diagnosis. Animals were subject to an OvSynch synchronization protocol. Blood samples were collected on d −10, 1 to 7, 10, 14, 18, 21, 24, and d 28 relative to breeding d 0 for P4 analysis. If animals showed heat after the first insemination they were rebred and were sampled for P4 on the same schedule as the first AI excluding d −10 and d 1 to 7 relative to the second AI. Pregnancy diagnosis occurred

at d 28 post-AI. Pregnancy rates were analyzed using a logit model with fixed effect of treatment and a random effect of pasture. Progesterone concentrations were analyzed independently using a mixed model with fixed effect of treatment, time, their interaction, and initial P4. A random effect of pasture was also included. Conception rate at first AI was not affected by treatment (46.67% and 50.00%) but overall conception rate was higher for starch fed than for fat fed heifers (83.33% and 61.54%). Despite lower overall conception rates, heifers on the fat supplement had significantly higher P4 concentrations ($P < 0.001$). Additional assessment of the mechanism by which diet influences conception rates is warranted

Key Words: cattle, reproduction, nutritional supplementation

Ruminant Nutrition General

279 Relationship between urinary energy and N excretion in lactating Jersey cows. D. L. Morris*[1], J. L. Firkins[2], W. P. Weiss[3], and P. J. Kononoff[1], [1]*Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE*, [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH*, [3]*Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.*

Estimation of urinary energy (UE) excretion is essential to determine metabolizable energy supply. Because most energy containing compounds in urine also contain N (e.g., urea, allantoin, hippuric acid, creatinine), our objectives were to validate the use of urinary N (UN) to estimate UE. Individual animal data from 4 studies ($n = 134$) were used. The data set included a wide range (min to max) in d in milk (88 to 346), dry matter intake (11.6 to 24.6 kg/d), N intake (282 to 642 g/d), energy-corrected milk yield (14.8 to 48.2 kg/d), UE excretion (1390 to 3160 kcal/d), and UN excretion (85 to 220 g/d). All models included the random effect of cow and period within study. The relationship between UE (kcal/g) and UN (g/100 g) was (parameter estimate ± standard error) $\text{UE} = 0.128 \pm 0.0074 \times \text{UN} + 0.0165 \pm 0.0054$. In the initial models between UE and UN, the intercept was 880 kcal/g of N for the linear model and not different from 0 ($P = 0.50$) for the quadratic model. Because most energy compounds in urine contain N, an intercept of 880 is biologically unlikely. The intercept was forced through 0. The regressions of UE (kcal/d) on UN (g/d) were $\text{UE} = 14.6 \pm 0.32 \times \text{UN}$ and $\text{UE} = 20.9 \pm 1.0 \times \text{UN} - 0.0357 \pm 0.0056 \times \text{UN}^2$. Slope bias was observed in the linear ($P < 0.01$), but not in the quadratic regression ($P = 0.19$). With increasing UN in the quadratic regression, UE increased but at a diminishing rate. Increasing UN from 100 to 150 g and 150 to 200 g increased UE by 599 and 420 kcal, respectively. To better understand the relationship between UE and UN, UE as kcal/g N was regressed against UN (g/d). The regression was $\text{UE (kcal/g N)} = -0.0412 \pm 0.0060 \times \text{UN (g/d)} + 21.8 \pm 1.0$. The ratio of UE to UN at 100, 150, and 200 g of UN excretion was 17.7, 15.6, and 13.6 kcal/g N, respectively. This decrease was likely due to an increase in the proportion of UN that is from urea which has a lower enthalpy per g of N compared with the non-urea components of urine (5.4 vs. 24 kcal/g N). Our analyses show that a quadratic relationship exists between UE and UN excretion from 85 to 220 g/d which is likely due to the dilution of non-urea N with increased UN excretion.

Key Words: metabolizable energy, bomb calorimetry

280 Effects of enzyme extracts from *Aspergillus oryzae* and *Aspergillus niger* on lactational performance of dairy cows fed a decreased-energy diet. M. E. Fetter*[1], S. E. Räisänen[1], C. de Assis Lage[1], H. A. Stefenoni[1], A. Melgar[1], S. F. Cueva[1], D. E. Wasson[1], D. M. Paulus Compart[2], and A. N. Hristov[1], [1]*Department of Animal Science, The Pennsylvania State University, University Park, PA*, [2]*PMI, Arden Hills, MN.*

The objective of this study was to investigate the effects of enzyme extracts from *Aspergillus oryzae* and *Aspergillus niger* (ENZ) on lactational performance and blood metabolites of Holstein cows fed a diet in which corn grain (8.6% of dry matter; PCON diet) was replaced with wheat straw (NCON and ENZ diets). Following a 2-wk covariate period, 48 multi- and primiparous cows averaging (±SD) 151 ± 45 d in milk (DIM) and 593 ± 55.7 kg BW were blocked based on DIM, milk yield (MY) and parity and randomly assigned to 1 of 3 treatments in an 8-wk randomized complete block design experiment: (1) positive control diet (PCON; 0.6 Mcal/d NE_L balance, based on NRC, 2001), (2) negative control (NCON; −1.4 Mcal/d NE_L balance) and (3) NCON supplemented with ENZ at 113 g/d per cow (−1.3 Mcal/d NE_L balance). Data were analyzed using PROC MIXED of SAS with block and block × treatment as random effects. Compared with PCON (24.0 kg/d), dry matter intake (DMI) decreased ($P = 0.03$) in both NCON and ENZ diets (22.5 and 22.1 kg/d, respectively). MY was also decreased ($P < 0.001$) by NCON and ENZ (32.0, and 30.8 kg/d, respectively) compared with PCON (35.4 kg/d). Milk fat concentration, but not yield, was greater ($P = 0.001$) for NCON and ENZ, compared with PCON (4.14, 4.08, and 3.76%, respectively). Compared with PCON, milk lactose concentration and yield and true protein yield were decreased ($P < 0.001$) by NCON and ENZ and so was energy-corrected milk yield (ECM; $P = 0.01$). Feed efficiency and ECM feed efficiency were not affected ($P \geq 0.51$) by diet. Cows gained less ($P = 0.01$) BW and blood glucose concentration tended to be lower ($P = 0.06$) for NCON and ENZ compared with PCON. The ENZ diet had no effect on milk yield and composition, feed efficiency, BW change, and blood metabolites compared with NCON. In this experiment, replacing corn grain with straw decreased DMI, MY, and milk true protein and lactose concentrations but increased milk fat concentration and did not affect feed efficiency in dairy cows. The enzyme extracts had no effect on the lactational performance of the cows.

Key Words: starch, fiber, dairy cow

281 New perspectives for metagenomic analysis: Rumen sampling via esophageal tube using a manual vacuum pump. F. E. Miccoli*[1,2], S. Ferreyra[1], S. Maresca[3], S. López-Valiente[3], P. Sueldo[3], N. Juliano[2], R. A. Palladino[1,4], J. L. Danelón[2], and R. I. Galarza[3], [1]*Faculta de Ciencias Agrarias - UNLZ, Buenos Aires, Argentina*, [2]*Departamento de Produccion Animal, UBA, Buenos Aires, Argentina*, [3]*INTA Cuenca del Salado, Buenos Aires, Argentina*, [4]*Consejo de investigaciones Científicas, CONICET, Buenos Aires, Argentina.*

Even though rumen fistulation is the main technique for rumen sampling, similar microbiome can be found between cannula and the intraesophageal tube samples. The main constrains of the tube technique are related to saliva contamination, comparable solid and liquid fractions and the site of extraction which affects the microbial community structure. With the aim to collect adequate samples for metagenomic analysis, a combined device + technique (Rumen Sampler MG) is described after validated with beef heifers (172.3 ± 16.17 kg BW) and dairy cows (554.66 ± 25.19 kg BW). Device: A manual pump (Professional Hand-held vacuum pump - Eurotech, Germany) is connected to a PVC flexible tube (40mm wide) through a short PVC tube (20 × 80 mm) to ensure the ideal vacuum. The intraesophageal tube is twice wider than other devices and has no holes to avoid obstructions with solid material and is beveled edge for a more rapid flux. Its length depends on animal size (the length to reach the rumen was previously checked using an endoscopic camera). Technique: Intravenous application of neuroleptic dosed at 0.25mL/100 kg BW (Acepromacina 10mg/mL, Acedan – Holliday, Argentina) and wait 3–5 min for the animal to relax and stay still. A rigid PVC tube (6x20cm) is located over the tongue to insert the flexible tube and past the epiglottis. Once inside the rumen, slight vacuum pressure is applied (7–10 bar) to avoid damages on the epithelium. After the sample is obtained, rumen contents are deposited in a 250mL beaker for visual examination of saliva contamination and pH measurement (Hanna HI98128 pHep 5 Waterproof), in addition to evaluate the liquid-solid ratio and total volume. In case the sample quality is not appropriate for metagenomic analysis, the sampling can be immediately repeated due to neuroleptic effect lasts between 15 and 17 min and the procedure takes approximately 8 min. The ´Rumen sampler MG´ combines a simple and rapid procedure and a more adequate device for obtaining higher quality samples for microbiome studies, in addition of taking care of animal welfare.

Key Words: rumen microbiome, esophageal tube, manual pump

282 Phytogenics improved colostrum quality when fed to close-up cows. E. Schwandt*[1], P. Gott[1], A. Tacconi[2], R. Murugesan[1], and S. Ramirez[1], [1]*BIOMIN America Inc, Overland Park, KS,*

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The anti-inflammatory properties of phytogenics may aid in diverting energy and nutrients toward productive biological processes, such as producing colostrum, by keeping the immune response homeostatic. The study objective was to determine the effect of a phytogenic feed additive, Digestarom Dairy (DD; BIOMIN America, Overland Park, KS) on colostrum quality when fed to close-up cows. One commercial close-up pen was utilized where cows entered this pen approximately 30 d before their calving date. A total of 641 cows received a control diet (CON) and 524 cows received a diet containing 2 g/h/d DD over 2 replicate periods per treatment group. Lactation groups were categorized as 2, 3, and 4+. Colostrum (kg) was collected, weighed, and Brix (%) measured via refractometer. Colostrum and Brix were analyzed via GLIMMIX procedure of SAS with a covariate of replicate and a random factor of cow; LSMeans represent treatment estimates within lactation. Brix was categorized as > 21% and ≤ 21% to represent higher quality colostrum (HIGH) and lower quality colostrum (LOW), respectively. The distribution of HIGH and LOW within CON and DD for each lactation and overall lactations were analyzed via Chi-squared test in the FREQ procedure. Colostrum was reduced ($P < 0.05$) in lactation 2, 3, and 4+ groups fed DD compared with CON by 0.45, 0.18, and 0.32 kg, respectively. However, Brix was increased ($P < 0.05$) in lactation 2, 3, and 4+ groups fed DD compared with CON by 1.5, 1.7, 0.7% points, respectively. Averaged over lactation groups, DD reduced ($P < 0.05$) colostrum by 0.32 kg and increased ($P < 0.05$) Brix by 1.4% points compared with CON. The distribution of HIGH colostrum tended ($P < 0.10$) to increase in lactation 2 and 3 groups fed DD compared with CON, but did not differ in distribution between treatments for lactation 4+ group. However, averaged over lactation groups, the distribution of HIGH and LOW quality colostrum was significantly ($P < 0.05$) different between treatments, where DD had greater HIGH colostrum cows (32.8%) compared with CON cows (26.6%). In conclusion, DD reduced total colostrum produced, but increased colostrum quality as measured by Brix.

Key Words: colostrum, phytogenics, Brix

283 Dietary phytogenics affect milk production in Holstein dairy cows. S. Ramirez*[1], P. Gott[1], A. Tacconi[2], and R. Murugesan[1], [1]*BIOMIN America Inc*, *Overland Park, KS*, [2]*BIOMIN Holding GmbH*, *Getzersdorf, Lower Austria, Austria*.

The anti-inflammatory properties of phytogenics may help keep the immune system homeostatic allowing for a more efficient use of nutrients and energy toward productive biological processes such as milk production. The study objective was to determine the effects of a phytogenic feed additive, Digestarom Dairy (DD; BIOMIN America, Overland Park, KS) on milk production of a commercial dairy herd. Nine pens were allotted to a control (CON, n = 4 pens) diet or a diet (n = 5 pens) containing DD at a target intake of 2 g/h/d. For 186 d, cows were milked twice per day and test day information was collected monthly. The amount of cows for each lactation group (1, 2, 3, and 4+) was 828, 470, 224, and 117, respectively for CON and 796, 732, 370, and 133, respectively for DD. For each cow within pen, daily milk production was fitted to a 305-d Wood's lactation curve model. From this model, peak milk, 305-d total milk, and persistency were estimated for each treatment and lactation group (1, 2, 3, and 4+) and these data were analyzed via GLIMMIX procedure of SAS. Test day fat and protein were used to calculate energy-corrected milk (ECM) at the estimated peak day and total milk for 305 d. For lactation 1, DD increased ($P < 0.05$) peak milk by 1.76 kg and increased ($P < 0.05$) total milk and total ECM by 522.08 and 170.59 kg, respectively compared with CON. For lactation 2, 3, and 4+, there was no statistical difference in lactation 2, 3, and 4+ between dietary treatments; however, averaged across lactation groups, DD significantly increased ($P < 0.05$) peak milk and ECM at peak by 2.16 and 1.22 kg, respectively compared with CON. The numerical improvement in total milk and total ECM was driven by a significant ($P < 0.05$) reduction in protein and numerical increase in fat when cows were fed DD compared with CON. In conclusion, DD improved milk production both at peak production and estimated 305 d total milk compared with CON, but the extent of this improvement was dependent on lactation group.

Key Words: milk production, phytogenics, lactation curve

284 Effect of breed and parity on performance responses in early lactation dairy cows. G. I. Zanton*, *USDA-ARS, US Dairy Forage Research Center*, *Madison, WI*.

The objective of this study was to characterize breed and parity effects on production and feed efficiency (FE) in first (1P) and second (2P) parity Holstein (H) and Jersey (J) cows. Cows were randomly selected from the research herd based on expected calving dates, enrolled into the study at calving (n = 13/group, except for 1P-J cows where n = 12), and studied for 12 wk. Cows were housed in tie-stalls and individually fed a total mixed ration once daily (formulated to contain 28.5% NDF, 17% CP, and 27% starch), milked thrice daily, and had free access to water throughout the study. Feed intake and milk yield were measured daily, BW was measured and each milking was sampled for composition semiweekly, and body condition score (BCS) and structural measurements were collected during wk 1, 4, 8, and 12. Data were analyzed as a completely randomized design with a 2 × 2 factorial combination of independent variables and evaluated for main effect (breed = Br, parity = Pa) and interaction (Br × Pa) responses with $P < 0.05$ signifying significant differences. A Br × Pa interaction existed for initial BW and structural measurements (2P-H > 1P-H > 1P-J = 2P-J), whereas calving BCS was not different among groups (3.85 ± 0.44 SD). Maximum losses of BCS and BW (% of initial BW) were greater for 2P than 1P, but did not differ between Br. DMI was affected by Br × Pa with 2P-H > 1P-H > 1P-J = 2P-J, however Br was the only factor affecting DMI when expressed as a % of BW with J > H. Protein concentration was greater for J than H and lactose concentration was greater for H than J, whereas Pa was without effect on these responses. Fat concentration was affected by Br × Pa with 1P-J = 2P-J > 2P-H > 1P-H; the difference between 1P-H and 2P-H was most pronounced before wk 7. Yields of fluid milk, components, and net energy were greater for H than J and for 2P than 1P, although Br × Pa affected the magnitude of these differences among groups. FE (milk net energy / DMI) was unaffected by Br and greater for 2P than 1P although these differences disappeared after wk 7. Under these conditions, Br, Pa, and Br × Pa affected production responses, however FE was only transiently affected by Pa.

Key Words: breed, efficiency, parity

Extension Education

285 Tennessee producers' perceptions of dairy farm facilities and their future in the industry. A. Sen*, S. Schexnayder, D. Bilderback, and E. Eckelkamp, *University of Tennessee Institute of Agriculture*, *Knoxville, TN*.

Farm facilities impact cow comfort, production, farm profitability, and success. Our objective was to identify barriers to dairy producers' permanency in Tennessee (TN). Our sub-objective was to assess producers' perceptions of existing farm facilities' longevity, facility investments in the last 5 yr, and planned facility improvements over the next 5 yr. A survey was distributed electronically and by mail in August 2019. Data were collected on producers' decision to stay or exit the dairy industry in the next 5 yr and their perceived longevity, past investments, and investment plan or automation in parlor equipment (PE), milk cow housing (MCH), dry cow housing (DCH), calf housing (CH), young stock housing (YSH), manure handling and storage (MHS), and feed handling and storage (FHS). Ninety surveys were returned and used in the analyses representing 48% of TN dairy producers. The FREQ procedure in SAS 9.4 was used to calculate the frequencies. Univariable logistic regression was done to identify barriers to producers' permanency. Significance level was set at $P < 0.10$. Mean ± SD herd size was 250 ± 356 (dry + lactating; range: 14 to 2300). For all facilities, mean ± SD perceived longevity was ≤5 yr (30 ± 4% of respondents), 6 to 10 yr (32 ± 3% of respondents), 11 to 15 yr (18 ± 2% of respondents), and >15 yr (20 ± 5% of respondents). Over the last 5 yr, producers invested in PE (n = 56), MCH (n = 33), CH (n = 24), YSH (n = 14), MHS (n = 32), and FHS (n = 48) with 15 producers not investing in any improvements. Over the next 5 yr, the top 3 facility improvements were PE (n = 25), MHS (n = 20), and FHS (n = 18), with 20 respondents planning no improvements. Very few producers (12%; n = 10) planned to have automation in any facilities. Producers' who did not invest in MCH ($P = 0.01$), CH ($P = 0.03$), and MHS ($P = 0.09$) in the last 5 yr were more likely to exit the dairy industry. Although 62% of producers perceived facility longevity was <10 yr, ≤ 29% of respondents planned any facility improvements over the next 5 yr. Improved animal housing and manure management might increase dairy producers' permanency in TN.

Key Words: farm facilities, improvement, investment

286 Do educational farm tours reduce concerns of individuals with greater concern about how food is produced? T. A. Ferris[1], R. R. Peters*[2], E. A. Richer[3], R. G. Slattery[2], C. W. Anderson[2], M. J. Rupp[3], and K. M. Miller[3], [1]*Michigan State University*, *E. Lansing, MI*, [2]*University of Maryland*, *College Park, MD*, [3]*Ohio State University Extension*, *Wauseon, OH*.

Breakfast on the Farm educational tours were held for consumers in Maryland and Ohio in 2019 with an objective of determining if tours increase trust in farmers and modern food production. Using an exit survey, participants indicated their level of trust that farmers will do right in managing various aspects of dairy farms. Using a 5-pt scale from *Very low trust* to *Very high trust*, respondents provided their level of trust BEFORE and AFTER the tour to self-assess changes in trust. Individuals were asked if they purchase organically produced products on a 5-pt scale from *Never* to *Always*. Those reporting purchases of organic, *Never, Rarely, or Sometimes* (n = 324) were coded non-organic consumers, and those who responded *Very often or Always* (n = 105) were coded organic consumers. Mean level of trust for farmers *Caring for the environment* for non-organic consumers was 4.02 before vs. 4.49 after with an increase of 0.476 while organic consumers had a lower mean of 3.69 before vs. 4.37 after with a greater increase in trust of 0.682. Similarly, mean levels of trust for farmers *keeping milk safe* before and after were 4.30 vs 4.62 and 3.90 vs. 4.49 for non-organic and organic consumers, respectively. Likewise, means for *Caring for food-producing animals* before and after were 4.13 vs. 4.55 and 3.75 vs. 4.41 for non-organic and organic consumers; and for *Using antibiotics responsibly* before and after were 3.95 vs. 4.38 and 3.53 vs 4.22 for non-organic and organic consumers. All changes in means between before and after responses for non-organic and organic consumers were significant ($P = 0.0001$) with a paired-t-test. For organic consumers, means increased most for *Using antibiotics responsibly*. Differences in means between non-organic and organic consumers ranged from 0.33 to 0.48 before and 0.12 to 0.26 after with the greatest difference after for using GMOs responsibility. These tours increased trust significantly in both groups and although buyers of organic products may have different perspectives before their visit, their trust became closer to those who do not purchase organic.

Key Words: educational farm tour, consumer perception, organic consumers

Animal Behavior and Well-Being

287 Feeding behavior and performance of dairy cows in an automated milking system is related to personality traits. A. J. Schwanke*[1], K. M. Dancy[1], G. B. Penner[2], H. W. Neave[3], and T. J. DeVries[1], [1]*Department of Animal Biosciences*, *Guelph, ON, Canada*, [2]*Department of Animal and Poultry Science, University of Saskatchewan*, *Saskatoon, SK, Canada*, [3]*Ruakura Research Centre, AgResearch Ltd*, *Hamilton, New Zealand*.

A goal of dairy cattle management is to meet the nutritional requirements of individuals, but there is large variability in behavior and performance of cows. The objective of this study was to identify and associate personality traits with behavior and performance of cows milked in a free-traffic automated milking system (AMS) and determine if the response to concentrate allocation in an AMS is associated with those personality traits. Holstein cows (n = 15; 124 ± 53 DIM; parity = 2.7 ± 0.9) were exposed to 2 treatments in a crossover design with 2 consecutive 28-d periods: cows were provided a basal PMR with a pelleted AMS concentrate allowance of: 1) 3.0 kg/d (L-AMS) or 2) 6.0 kg/d (H-AMS). During the last 5 d of the first period, behaviors in response to a novel arena, object and human were scored to identify personality traits. Principal component analysis revealed 5 factors interpreted as personality traits (2 from novel human test = 72% cumulative variance; 3 from novel object and novel arena tests = 81% cumulative variance). Linear regressions were used to explore relationships between each factor and behavior and performance outcomes. Cows high on Factor 1 (active and vocal) had greater total DMI, sorting of the PMR, and lying bouts, but shorter lying bout duration ($P < 0.01$). Cows high on Factor 2 (fearful of human) had greater meal size ($P = 0.05$), gained BW ($P = 0.04$), and required more AMS fetches ($P < 0.01$), likely explaining their lesser concentrate intake and milk yield ($P < 0.01$). These cows were less likely to meet their target concentrate allowance on the H-AMS treatment ($P < 0.01$). Cows high on Factor 3 (active) gained BW ($P = 0.05$) and had more problematic milkings ($P < 0.01$), likely explaining their lesser milk yield ($P = 0.02$). Cows high on Factor 4 (social) had greater meal frequency and SCC ($P < 0.01$) and lesser % milk fat ($P = 0.03$) and protein ($P = 0.04$). Cows high on Factor 5 (fearful of object) had greater eating rate ($P = 0.02$), rumination ($P = 0.03$), and % milk protein ($P = 0.01$). These results indicate that personality traits of dairy cattle are associated with feeding behavior and performance in an AMS.

Key Words: temperament, robotic milking system, individual variation

288 Repeated regroupings affect body and reproductive development and energetic metabolism. M. Moratorio*[1], A. Amil[1], M. Pedrozo[1], R. Ungerfeld[2], M. Carriquiry[3], and C. Fiol[1], [1]*Departamento de Bovinos, Facultad de Veterinaria, Universidad de la República*, *Montevideo, Uruguay*, [2]*Departamento de Fisiología, Facultad de Veterinaria, Universidad de la República*, *Montevideo, Uruguay*, [3]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República*, *Montevideo, Uruguay*.

The aim was to determine the effects of social regrouping (SR) on body and reproductive development and energetic metabolism in dairy heifers. Holstein heifers (153.3 ± 16.1 kg, 11 mo old) were allocated to 2 homogeneous groups according to BW, age and farmer origin: 1) Control (CON; n = 14 + 5 “fixed”) and 2) Regrouped (RG; n = 14 + 5 “exchanged”), in which “exchanged” were switched for 5 unknown heifers every 21 d (total = 10 SR; Day 0 = SR 1). In each SR the BW, ADG, withers height (WH) and BCS were registered, blood samples were taken for IGF-1, glucose, NEFA and albumin determinations. In addition, date at first heat was registered. Body development, IGF-1 and metabolites data were analyzed by PROC GLIMMIX, and days to first heat were compared with PROC Lifetest. Body weight and ADG changed across SR (Table 1). Control heifers had greater WH and BCS in SR 8 (122 vs 120 cm ± 1.8), 9 (123 vs 120 cm ± 1.8) and 10 (124 vs 122 cm ± 1.8), and in SR 4 (3.5 vs 3.2 ± 0.15) and 5 (3.4 vs 3.1 ± 0.15), respectively, than RG. Insulin growth factor-1 concentration was greater in CON than RG heifers (Table 1), and CON had greater IGF-1 concentrations in SR 3 (207.4 ± 20 vs 97.4 ± 12 ng/mL), 5 (184.5 ± 17 vs 97.4 ± 12 ng/mL), 7 (175.9 ± 17 vs 122.4 ± 14 ng/mL) and 9 (193.5 ± 18 vs 137 ± 15 ng/mL) than RG. Glucose and albumin concentrations of CON were greater than RG heifers (Table 1), and glucose was higher in CON than RG heifers in SR 3 (4.7 vs 4.4 mmol/L ± 0.1) and 6 (4.2 vs 3.7 mmol/L ± 0.1). In contrast, NEFA was greater in RG than CON heifers (Table 1), and RG had greater NEFA than CON in SR 3 (0.6 ± 0.09 vs 0.3 ± 0.06 mmol/L), 5 (0.5 ± 0.08 vs 0.3 ± 0.05 mmol/L) and 7 (0.4 vs 0.3 mmol/L ± 0.05). Control heifers showed first heat earlier than RG heifers (93 ± 9 vs 126 ± 14 d; $P < 0.05$). In conclusion, frequent SR negatively affected body and reproductive development and energetic metabolism.

Table 1 (Abstr. 288).

	Group			*P*-value		
Item	CON	RG	SEM	Group	SR	Group × SR
BW, kg	266.7	259.9	5.4	ns	<0.01	ns
ADG, kg/d	0.82	0.79	0.02	ns	<0.01	ns
WH, cm	115.9	115.4	1.8	ns	<0.01	0.01
BCS	3.4	3.3	0.13	ns	<0.01	<0.01
IGF-1, ng/mL	177.4	116.5	10.5	<0.01	0.02	<0.01
Glucose, mmol/L	4.3	4.2	0.07	0.04	<0.01	0.05
Albumin, mmol/L	30.0	28.6	0.6	0.01	<0.01	ns
NEFA, mmol/L	0.30	0.35	0.04	0.04	<0.01	<0.01

Key Words: social regrouping, heifer

289 Impact of a dietary citrus extract on the rumination behavior of cows following social regrouping. F. H. Padua*[1], R. Bergeron[1], G. Desrousseaux[2], J.-F. Gabarrou[2], and T. J. DeVries[1], [1]*Department of Animal Biosciences*, *Guelph, ON, Canada*, [2]*Phodé*, *Terssac, France*.

The objective of this study was to determine if feeding a citrus extract (CE; derived from *Citrus sinensis*) reduces the negative impact of social regrouping of lactating dairy cows. It was hypothesized that cows supplemented with CE would demonstrate a quicker stabilization of their rumination behavior after being moved into a new group of cows. Thirty-two multiparous (parity = 2.1 ± 0.1) mid-lactation Holstein dairy cows (169.8 ± 1.1 DIM) were enrolled as focal cows in this study and housed individually a tie-stall facility where they were assigned to 1 of 2 treatment diets: 1) control TMR (control; n = 16), or 2) control TMR with 4 g/d of citrus extract (VeO; Phodé, Terssac, France) (CE; n = 16). Cows were fed their experimental diets for 7 d in the tie-stall facility (P1), then moved to 1 of 2 experimental freestall pens (containing 29 other cows) for a period of 7 d (P2), where they remained on the same treatment diet as provided in the tie-stall facility. This process repeated until all 16 cows/treatment were introduced to the group pens. Data were analyzed using repeated measures mixed-linear regression models to test whether rumination varied in P2 within cow, by day, from their P1 average. In P1, CE cows consumed 27.2 ± 0.4 kg/d of DM and ruminated for 490 ± 9 min/d, while control cows consumed 27.7 ± 0.4 kg/d of DM and ruminated for 505 ± 9 min/d. In P2, CE cows ruminated 537 ± 6 min/d, while control cows ruminated 519 ± 6 min/d. For control cows, rumination time did not change after regrouping ($P > 0.05$), while for CE cows, rumination time increased after d 2 ($P < 0.04$). A tendency for a difference in change in rumination time ($P = 0.09$) was identified on d 2 after regrouping, whereas control cows were reduced by 6.3 ± 21.3 min/d from their P1 average, while CE cows were increased by 46.3 ± 22.0 min/d from their P1 average. The results indicate that feeding a citrus extract additive to mid-lactation dairy cows may promote a quicker stabilization of rumination behavior after regrouping.

Key Words: citrus extract, social regrouping, behavior

290 Validation of an infrared camera for measuring ocular temperatures of veal calves.

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Temperature measurement is a key part of the clinical exam process, however, the standard method to monitor temperature using rectal temperatures is subject to errors and can be laborious and disruptive to animal behavior. Use of infrared thermography (IRT) is a plausible alternative to rectal temperatures for providing a non-invasive method to assess calf health. The objective of this prospective cohort study was to validate IRT for measuring core body temperatures. A total of 320 calves were enrolled upon arrival at a veal facility in southwestern Ontario, Canada. Calves were followed for 14 d between May and August 2019. Researchers visited the farm daily to measure ocular infrared (IR) temperature and rectal temperature (RT), as well as evaluate navel, attitude, fecal, and respiratory scores. The IR camera was placed at a distance of 12 inches away from the calf's eye to ensure consistent measurement. Treatment and mortality records were also collected throughout the 78 d the calves were at this facility. The mean difference in IR temperature and RT was 0.30°C ± 1.50. Youden's Index was used to determine the optimal cutpoint which would maximize the sensitivity and specificity of the IR camera for detecting a fever when compared with a RT of ≥39.5°C. The optimal cutpoint for the infrared camera to detect a fever, defined as a RT of ≥39.5°C, was 39.45°C, and the sensitivity and specificity of detecting a fever using IRT at this point were 60% (95% Confidence Interval (CI): 53, 67) and 71% (95% CI: 70, 73), respectively. The area under the receiver operating characteristic (ROC) curve at this point was 0.66 (95% CI: 0.62, 0.69). A random number generator was used to select a day between 1 and 14, and a simple linear regression model was built to assess the ability of the IR camera to predict RT on d 10. The R squared of this model was 0.0122, suggesting that IR temperature alone was poorly correlated with RT. Further analysis is being conducted to explore external variables which influence accuracy of the IR camera and to evaluate the predictive ability of IRT in assessing calf health.

Key Words: male dairy calf, morbidity

291 Effect of two stable fly control methods on dairy cattle bunching behavior on a California dairy.

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The stable fly (*Stomoxys calcitrans*) is blood-feeding fly that targets the lower limbs and abdomen of cattle leading animals aggregating into groups, a protective behavior known as bunching. The objective of this crossover study was to evaluate the effectiveness of 2 stable fly control methods on reducing the incidence of bunching and stable fly counts on the front legs of cows. The study was conducted on 3 pens, of approximately 150 lactating cows each, at a single dairy over 3 replicated phases. Pens were assigned to the following treatments: 1) KattleGuard fly spray system (KG) which dispenses a solution of 1% permethrin and 1% piperonyl butoxide over the back and legs of cows as they exit the milking parlor, 2) trigger fabrics impregnated with 0.1% lambda-cyhalothrin (TF) hung along the feedbunk of the pen, or 3) a no treatment control (CON). The treatment period lasted for 10 d, followed by a 4-d washout period before the treatments were rotated among the same 3 pens. Stable fly counts on cow legs and cattle bunching in the pens were recorded twice per day (9–11 and 12–2). Bunching was recorded daily at the pen level and stable fly counts were recorded on 15 cows from each pen by 2 trained personnel. The log-transformed mean number of flies on the legs of 15 cows from each pen was calculated and analyzed with pen and period as crossed random effects in a linear mixed model. Bunching was analyzed using mixed-effects logistic regression. Cows treated with KG had significantly reduced odds of bunching during the afternoon (OR = 0.002; $P = 0.001$) in comparison to CON pens. Similarly, TF reduced the odds of bunching during the afternoon times (OR = 0.04; $P = 0.01$) in comparison to CON. During afternoons, cows treated with KG had ($P = 0.03$) lower stable fly counts on their legs (0.67 ± 0.21 flies/legs) in comparison to untreated group of cows (1.11 ± 0.20 flies/legs); however, the fly count on legs in TF pens (1.00 ± 0.30 flies/legs) was not different from CON ($P = 0.67$). The current study showed that the fly spray system significantly reduced bunching and stable fly biting during the afternoon on a dairy in Tulare County.

Key Words: fly spray, treated fabrics, stable fly

292 Daily milk losses associated with bunching, dairy cattle's protective behavior against stable flies (*Stomoxys calcitrans*), on a California dairy.

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Bunching is the behavioral phenomenon of cattle aggregating in tight groups to protect them from biting by stable flies (*Stomoxys calcitrans*). The incidence of bunching varies between dairies and pens within the same dairy as it is associated with the intensity of stable flies on the dairy, management and environmental factors. In addition, bunching may be associated with heat stress experienced by cattle aggregation, as well as a decrease in feeding and laying times. Thus, bunching may affect dairy cows' milk production mediated through a reduction in dry matter intake and rumination times. However, there are no previous studies on the effect of bunching on milk production in lactating dairy cows. The objective of our study was to estimate the impact of cow bunching on milk production on a commercial dairy. A longitudinal study was conducted between April 26th, 2017 and July 31st, 2017 on a large Holstein herd housed in freestall pens in Tulare, California. The study dairy used the KattleGuard fly spray system (Dairy Solutions Inc., Tulare, CA) as a fly control program. Pen level cow bunching was recorded weekly on 4 lactating cow pens for 13 weeks. Bunching observations were matched to daily milk records from the study's 2 high production pens (558 cows) and 2 low production pens (591 cows) by day of observation. Two-piece spline linear mixed models were used to estimate the association between cow bunching and milk production. On average, bunching was associated with a decrease in daily milk production of 2.72 kg ± 0.486 per cow ($P < 0.01$). In addition, cows in high producing pens had 2.68 kg ± 0.302 more daily milk production compared with cows in low producing pens. Additionally, the model showed that, compared with first lactation cows, there was a significant increase in milk production in second lactation cows (8.92 kg ± 0.564) and third or greater lactation cows (10.71 ± 0.564 kg). Bunching due to stable flies can negatively affect dairy cattle welfare and productivity.

Key Words: bunching, daily milk, stable flies

Breeding and Genetics

293 Methods to implement ancestor discovery in the US dairy cattle database. J. Nani*[1,2], J. Cole[2], and P. VanRaden[2], [1]*Instituto Nacional de Tecnologia Agropecuaria EEA Rafaela, Rafaela, SantaFe, Argentina*, [2]*Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA, Beltsville, MD.*

Accurate and complete pedigrees are, even in the post genomics era, fundamental to plant and animal breeding because accurate genetic and genomic evaluations often rely on making genomic and pedigree relationships consistent. Program Fixped uses haplotypes to accurately confirm or discover distant relatives, such as maternal grandsires (MGS) and maternal great-grandsires (MGGS), with improved efficiency compared with individual SNP methods. In the US dairy cattle database, around 300,000 animals with no dam ID can be linked to their discovered MGS and MGGS by creating a constructed dam or maternal granddam (MGD) ID to fill in the missing pedigree information. Program Finddam creates the constructed dam and/or MGD ID to link calves to MGS and MGGS in the pedigree. This ID consists of 3 parts: 1) as currently, a 3-letter country code so that each country can construct their own IDs (i.e., USA), 2) DAM or MGD following the country code for the calf dam and maternal granddam respectively, and 3) the numeric portion of the calf ID (animal key) to ensure stability of data processing that can be expanded to 9 digits in the next few years. Program Finddam also allows linking calves to ~60,000 discovered MGS and MGGS not previously added because their dam and MGD ID were already reported. Recently expanded features of Fixped are discovery and confirmation of close relatives such as sires, dams, full and half sibs, clones, and paternal grandsires. Implementation of Fixped will increase the speed of genotype loading and avoid processing delays near deadlines because the current uploading program can then only confirm if the reported ancestors are correct and avoid searching the whole database for genotypes of relatives. Finally, when a real dam ID is found outside the database, those IDs will be preferred over constructed IDs unless the reported dam or MGD do not match the genotypes of the calf and grandsire. Pedigree providers will have an option to remove discovered relationships that they believe to be incorrect.

Key Words: ancestry discovery, pedigree, genomics

294 Bias of dairy sheep evaluations using BLUP and single-step genomic BLUP with metafounders and unknown parent groups. F. L. Macedo[1,2], O. F. Christensen[3], J. M. Astruc[4], I. Aguilar[5], Y. Masuda[6], and A. Legarra*[1], [1]*INRA, Toulouse, France*, [2]*UdelaR, Montevideo, Uruguay*, [3]*Aarhus University, Aarhus, Denmark*, [4]*IDELE, Toulouse, France*, [5]*INIA, Montevideo, Uruguay*, [6]*University of Georgia, Athens, GA.*

Bias is a problem in pedigree-based and genomic-based predictions, and it hampers correct selection procedures. Assessing bias for small dairy cattle breeds, sheep, and goat is difficult. Also, there is a plethora of options to integrate Unknown Parent Groups in Single Step GBLUP. In this work we quantify possible biases in predictions for a dairy sheep breed (Manech Tete Rousse). This breed has a selection scheme for milk yield based on performance recording, progeny testing and Artificial Insemination (AI). The data comprises ~35 years, 1,842,295 performance records and 540,999 individuals in pedigree. In pedigree, there are 70% animals with sire and dam known, 15% with missing sire and 15% missing both. We defined 13 Unknown Parent Groups (or Metafounders). 3007 AI males were genotyped with 50k SNP chip. We tested models with and without genomic information (BLUP and SSGBLUP) and using 3 strategies to handle missing pedigree (Unknown Parent Groups (UPG), "Exact" UPG (EUPG), and Metafounders (MF). The Gamma relationship matrix across MF was estimated by GLS from genotypes of rams and showed mild correlation across MF. To quantify bias, we used method LR. We generated "partial" data deleting most recent records at every year from 2005 to 2014. Then we created "whole" data deleting records with cut-off years from 2007 to 2017. Then we compared (G)EBVs from "partial" and (G) EBVs of young rams from "whole" data across several pairs of cutoff dates, resulting in 65 comparisons. All models resulted in some overestimation of the genetic trend of 0.20 – 0.40 genetic standard deviations. As for the slope (over/underdispersion of (G)EBVs) BLUP_MF, BLUP_UPG, SSGBLUP_MF and SSGBLUP_UPG were unbiased (slopes near 1 with s.e. ~0.02 across comparisons) whereas SSGBLUP_EUPG was biased (slope 0.87 with s.e. 0.02). This is probably due to double counting. One particular truncation year (2008) showed bias for all methods (~0.70 for SSGBLUP_MF and ~0.90 for the other methods) and the likely reason was suboptimal collect of young males that particular year.

Key Words: genomic, bias, sheep

295 Parent and grandsire discovery in a rapidly expanding collection of genotypes. G. Wiggans*, *Council on Dairy Cattle Breeding, Bowie, MD.*

For genomic selection based on SNP, the Council on Dairy Cattle Breeding (Bowie, MD) has collected over 3.9 million genotypes. In 2019, over 67,000 genotypes were added monthly on average. To assure that a genotype is assigned to the correct animal and that the pedigree is correct, parents are verified, and each genotype is compared with other genotypes to detect unreported parents or progeny or a duplicate genotype. To speed this discovery, a set of 4,668 SNP was defined based on their presence on nearly all genotyping chips, parent-progeny consistency, and minor allele frequency. Assessment is done after 96 and 1,000 SNP so that comparison can stop if a relationship is unlikely. If both parents are confirmed, only genotypes from potential relatives born < 500 d earlier are checked to detect duplicates. Each SNP genotype is represented by 2 bits rather than 1 byte to save storage space. Because discovered relationships are recorded based on genotype-specific identification, they are unaffected by the assignment of the genotype to a different animal. This process improves efficiency by comparing 2 genotypes only once, using sequential memory access when doing comparisons, and limiting discovery to just once a day to reduce setup time. The design allows use of the presence of progeny and date loaded to exclude comparisons with genotypes unlikely to be related. When a parent is not confirmed, the grandsire may be designated as unlikely using the same SNP set. For unlikely and unknown grandsires, discovery is done weekly based on haplotype matching, which relies on imputation done for weekly evaluations. This haplotype analysis also discovers other relationships, which can provide a check on SNP-based discovery. These changes in discovery method were developed to address the ever-increasing computing time needed as the number of genotypes in the US genetic evaluation system rapidly grows. The new discovery design is expected to enable a continued high level of genotype validation and relative discovery for many years as the genotype collection expands.

Key Words: dairy cattle, parentage discovery, genotype validation

296 Profiles of causative SNP in a genome-wide association study. I. Misztal*[1], I. Pocrnic[1,2], M. Perez-Enciso[3], and D. A. L. Lourenco[1], [1]*University of Georgia, Athens, GA*, [2]*The Roslin Institute, Midlothian, United Kingdom*, [3]*CRAG, Barcelona, Spain.*

The purpose of this study was to see the impact of causative SNP on GWAS with different populations with different effective population size. Three populations were simulated assuming 100 equidistant causative SNP with identical substitutions effects. Causative SNP were included in 50 k SNP genotypes. Ten generations were simulated, with the last 3 genotyped. Population NE60 was composed of 2000 animals per generation with effective population size 60. Population NE600 was composed of the same number of animals but with effective population size 600. NE60_3x was as NE60 but with 6000 animals per generation. Analyses were performed using single step GBLUP, with solutions converted to SNP values and subsequently to p-values for each SNP; in a GBLUP context, p-values are equivalent to those in standard GWAS methodology, where each SNP is treated as fixed effect, and a genomic relationship matrix accounts for the population structure. Manhattan plots for standardized SNP solutions showed large values for few of the 100 causative SNP and were very noisy. Manhattan plots for p-values were similar to those for SNP solutions. The number of SNP effects with p-values over the statistical threshold was smallest for NE60, larger for NE60_3X, and the largest for NE600. SNP profiles were created by averaging SNP solutions ± 100 SNP around causative SNP. The profiles showed distinct peak for the causative SNP, with smaller signals for adjacent SNP. The peak was smallest for NE60 and largest for NE600. Each causative SNP influenced about 50 adjacent SNP for NE60 and NE60_3X, and about 10 SNP for NR600. The profiles help understand that the effective use of causative SNPs requires knowing their exact positions and either boosting their variance in analyses or elimination of SNPs adjacent to causative SNPs.

Key Words: genomic selection, causative SNP, sequence data

297 Predicted producing value: Formula to account for actual inbreeding in a mating program framework.

S. Westberry*, C. Heuer, N. Deeb, and D. Kendall, *STgenetics*, *Navasota, TX.*

Increasing herd profitability is essential to dairy producers desiring to withstand the ever-changing dairy industry climate. When optimizing future herd performance based on parent average, a crucial piece of the mating value is missing. The actual relationship of each mating pair needs to be determined and how the resulting inbreeding will affect the producing value of the progeny. In the US, PTA evaluations produced by the CDCB are penalized for expected inbreeding depression based on the Expected Future Inbreeding (EFI) of the animals. The Predicted Producing Value (PPV) formula first removes the inbreeding penalty from sire and dam PTA values. The next step of the formula is to determine the expected progeny inbreeding or actual relationship of each mating pair which utilizes the genomic relationship between all pairs in the proposed matings. The expected inbreeding of the calf can then be multiplied by the inbreeding depression of the selected trait for optimization by utilizing the inbreeding depression factors published by the USDA. In a recent study, STgenetics compared utilizing a parent average PTA optimization mating program and a PPV optimization mating program in Chromosomal Mating. The mating scenario included optimizing Lifetime Net Merit (NM$) across 26,500 females and 100 bulls. The $\text{PPV}_{\text{NM\$}}$ of the projected progeny was calculated for both optimization strategies so that the 2 results could be compared. The mating that optimized $\text{PTA}_{\text{NM\$}}$ had an average progeny $\text{PPV}_{\text{NM\$}}$ of 1604, while the mating that optimized $\text{PPV}_{\text{NM\$}}$ had an average progeny $\text{PPV}_{\text{NM\$}}$ of 1631. This means that optimizing for $\text{PPV}_{\text{NM\$}}$ increased the value of the progeny by $27 over the lifetime of the progeny on average. The mating that optimized $\text{PPV}_{\text{NM\$}}$ also yielded projected progeny with 1.3% lower inbreeding on average compared with the projected progeny from optimizing $\text{PTA}_{\text{NM\$}}$. Utilizing PPV to optimize progeny value in a mating program allows producers to make the most accurate mating decisions based on profitability.

Key Words: inbreeding, mating program, genomic selection

298 Inbreeding depression due to different age classes of inbreeding on production and fertility traits in Canadian Holsteins.

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The reduction in the mean phenotypic performances of livestock animals could be attributed to rising inbreeding levels. However, this observed decline may not be caused by the total number of inbreeding. Therefore, partitioning inbreeding into different age classes could help in assigning detrimental effects to different classes. The aim of this study seeks to investigate the effect of recent and ancient inbreeding on production and fertility traits in Canadian Holstein cattle. Phenotypic records of 46,430 cows with birth year ranging from 2007 to 2017 were available for production and fertility traits. These animals had 50K genotype data and pedigree records, which comprised of 259,871 individuals. Inbreeding coefficients were estimated using traditional pedigree measures (*FPED*) and genomic pedigree measures using segment-based (*FROH*) and marker-by-marker (*FGRM*) based approaches. Additionally, both pedigree and genomic inbreeding were partitioned into different classes by tracing the pedigree back to a specific generation and using the specific length of homozygous segments to represent different classes, respectively. Inbreeding depression was found for all production and most fertility traits, for example, every 1% increase in *FPED*, *FROH* and *FGRM* was observed to cause a −44.71, −40.48 and −48.72 kg reduction, respectively, in 305-d milk yield. Similarly, an extension in the first service to conception (FSTC) of 0.29, 0.24 and 0.31 d in heifers was found for every 1% increase in *FPED*, *FROH* and *FGRM*, respectively. Partitioning both pedigree and genomic inbreeding into age classes resulted in recent age classes showing more unfavorable inbreeding effects, while more distant age classes caused a more favorable effect. For example, we observed a −1.56 kg loss in 305-d protein yield for every 1% increase in the most recent pedigree age class, whereas 1.33 kg gain was found per 1% increase in the most distant pedigree age class. In this study, we found heterogeneous effect of inbreeding by partitioning inbreeding into different age classes.

Key Words: inbreeding depression, recent and ancient inbreeding, pedigree and genomic inbreeding

Dairy Foods Cheese

299 Impact of milk fat globule membrane materials on cheese made from reconstituted milk: Structure and volatile organic compounds. H. Zheng*[1,2], M. Arnold[2], K. Kilcawley[3], T. Harding[4], and M. Weststeyn[2], [1]*Department of Food, Bioprocessing and Nutrition Sciences, Southeast Dairy Foods Research Center, North Carolina State University*, *Raleigh, NC*, [2]*Dairy Innovation Institute, Animal Science Department, College of Agriculture, Food and Environmental Sciences, California Polytechnic State University*, *San Luis Obispo, CA*, [3]*Teagasc Food Research Centre Moorepark*, *Fermoy, Ireland*, [4]*Department of Materials Engineering, College of Engineering, California Polytechnic State University*, *San Luis Obispo, CA*.

In this research, we aim to develop a technology for manufacturing high quality cheddar style cheese using fully reconstituted milk system made from dried milk components and water to mimic key structural and flavor attributes of cheese made from fresh milk (M). Cheese made from reconstituted milk are different than the cheese made from fresh milk in terms of textural characteristics and flavor profiles. By using microfiltration and spray drying, we manufactured a milk fat globule membrane (MFGM) enriched ingredient from buttermilk, the ingredient is named as buttermilk concentrate (BMC). BMC was used to emulsify butter fat droplet in the reconstituted milk system (RM-BMC) for mimicking the sizes, surface structure and composition of native milk fat globules found in the fresh cheese milk. Reconstituted milk (RM) made without BMC is the control. Laser diffraction, confocal microscopy, electron microscopy and electrophoresis studies confirmed that the oil droplets in RM-BMC system have similar characteristics in comparison with native milk fat globules. However, the xanthine oxidase (a MFGM protein) load on the surface of oil droplet is 50% lower in RM-BMC ($P < 0.05$). After 5-mo ripening, the microstructure of RM-BMC-cheese can mimic fresh milk cheese (M-cheese) in terms of protein gel network and fat droplet distribution. SPME-GC-MS technique was used for analyzing volatile compounds in cheeses after different ripening period (fresh, 1-, 3-, 5-mo), in total we identified 38 volatile compounds (VOCs) consisting of ketones (9), aldehydes (7), benzenes (5) acids (4), terpenes (4), sulfurs (3), alcohols (2), lactone (1) and furan (1). Principal component analysis (PCA) of VOC composition ratios showed encouraging results that, after 5-mo ripening, RM-BMC-cheese showed similarity to M-cheese in terms of VOC profile because PC2 (16.6%) groups these 2 type samples together and discriminates the control (RM-cheese) apart. Moreover, at the same ripening point, the flavor profile of M-cheese is very different than the control because they are well separated by both PC1 (49.3%) and PC2 (16.6%).

Key Words: cheese, milk fat globule membrane, buttermilk

300 Application of laser-induced breakdown spectroscopy technique for studying salt diffusion in model cheese matrices. P. Sharma*[1,3], M. Casado-Gavalda[2], J. Sheehan[1], and C. Sullivan[2], [1]*Teagasc Food Research Centre*, *Moorepark, Fermoy, Co. Cork, Ireland*, [2]*School of Food Science and Environmental Health, Technical University Dublin*, *Dublin, Ireland*, [3]*Utah State University*, *Logan, UT*.

Salt dissolved in the aqueous phase of cheese, plays vital roles during cheese manufacture by manipulating protein-protein and protein-water interaction and during ripening by contributing to the flavor of cheese, controlling the growth of microorganisms, and helping with enzymatic breakdown of cheese. Salt diffusion in the cheese moisture phase is a slow process. Conventional methods used for tracking salt migration within cheese matrices involve wet chemistry, tedious sample preparation and longer estimation times. Laser-induced breakdown spectroscopy (LIBS) is a novel technique which is currently used for mineral analysis in different food materials. The technique gives spatial distribution (at macroscopic level) of minerals (Na, K, Ca) on a surface; it requires no sample preparation; it is a non-invasive and quick method and it doesn't involve hazardous chemicals and procedures. The current work examined the ability of LIBS technique to capture sodium/salt migration in cheese systems. Cheese samples with different contact time (0, 30, 60 min) with brine (23%) solution were cut into half cubes (2.5x2.5x2.5 cm^3). Spatially distributed LIBS spectra were collected from interior cut surfaces/cross sections by applying laser shots in 45x45 square grid patterns. The emission peaks of plasma light at 589.05, 393.339 and 769.826 nm in each spectral data moment were used to generate sodium, calcium and potassium distribution images, respectively. A clear difference in spatial distribution of Na was observed in the control as compared with brined model cheeses dipped for 30 and 60 min. With brining time, the relative area of pixels indicating higher Na concentration levels increased significantly. Progressive diffusion of salt within model cheese matrix was clearly evident in spatial distribution mapping plots for Na. This work highlights a novel application of LIBS technique for generating spatially distributed salt concentration maps within cheese matrices. This technique may be applied for studying salt diffusion kinetics.

Key Words: laser-induced breakdown spectroscopy, salt diffusion, model cheese matrices

301 Modelling inward diffusion of salt in model cheese matrix using time-lapse confocal laser scanning microscopy. P. Sharma*[1,3], J. Sheehan[1], and J. Floury[2], [1]*Teagasc Food Research Centre*, *Moorepark, Fermoy, Co. Cork, Ireland*, [2]*STLO, INRAE*, *Agrocampus Ouest, Rennes, France*, [3]*Utah State University*, *Logan, UT*.

Salt dissolved in the aqueous phase of cheese, diffuses slowly through the dense protein network and influences structure, texture and flavor development. Although, cheeses may have similar gross composition, a hypothesis exists that differences in microstructure will result in different rates of salt migration. The objective of this study was to determine the influence of varying pH (5.4 vs 6.8) on the diffusion rate for salt in a model cheese system, i.e., renneted casein gels. Model cheese systems were prepared by adding rennet to a Micellar Casein Concentrate (~15% casein) after pH adjustment with glucono-δ-lactone and addition of protein (Fast Green; 1% wt/wt) and sodium (CoroNa green; 100µM) sensitive fluorescent dyes. Gels were formed in specialized slides (IBIDI) followed by brine addition (NaCl 23%). Salt diffusion was tracked using an inverted confocal laser scanning microscope (laser excitation at 488 nm). Microstructural parameters of both protein gels were quantified from confocal imaging (laser excitation for Fast Green at 633 nm) using the high-resolution detection unit (Airyscan). The effective diffusion coefficients for salt obtained after fitting data on Fick's second law were slower than previous reports. This was attributed to the absence of structural irregularities such as free water and fat channels in the isotropic dense hydrated protein (15%) network. Diffusion coefficients at pH 6.8 (6.44 ± 2.33 µm^2/s) were significantly ($P < 0.05$) lower than at pH 5.4 (18.64 ± 4.45 µm^2/s) and were attributed to a less porous gel structure at pH 6.8 (median pore size = 2.92 µm) compared with pH 5.4 (median pore size = 4.45 µm). Microstructural heterogeneity at the lower pH was attributed to a greater phase separation between protein and water as observed by CLSM. This novel salt tracking system suggests that varying cheese physico-chemistry influences salt diffusion rates. This technique will support further research particularly on diffusion behavior of salt in reduced moisture cheeses.

Key Words: model cheese, salt diffusion, confocal laser scanning microscopy

302 Effect of late lactation on the physicochemical and sensory properties of semi-hard goat cheese with reduced-fat content. F. Pinto[1], J. L. Riveros[2], and R. A. Ibáñez*[2,3], [1]*Pontificia Universidad Católica de Chile, Facultad de Agronomía e Ingeniería Forestal, Escuela de Graduados*, *Santiago, Chile*, [2]*Pontificia Univer-*

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The dairy market has experienced an increase in consumers interest for healthier products, particularly in cheeses with reduced fat content. On the other hand, lactation stage (LS) has a direct influence on the composition of milk that may impact the properties of cheeses. However, little attention has been focused to the effects of late LS on the composition and quality of goat cheese. We believe that a standard cheese manufacture protocol based on the composition of milk has a little impact on the properties of goat cheese with varying fat levels. In this study, the relationship between the chemical, textural sensory properties of full- (FF) and reduced-fat (RF) semi-hard goat cheeses made with milk from late LS were analyzed. Goat milk at 170, 190, and 210 d of lactation were collected from a local farm to produce milled-curd FF and RF cheeses ripened for 90 d at 10°C. As expected, FF and RF cheeses had differences in composition ($P < 0.05$), which directly impacted their physicochemical and sensory properties. In contrast, LS had no impact ($P > 0.05$) on most of the components of cheeses. Cheeses made from late LS had decreased levels of lactic acid, which also led to increased pH values ($P < 0.05$), probably due to a reduction in the lactose content in milk at late LS. Increasing LS led to cheeses with reduced proteolysis (mg leucine/100 g cheese estimated by the trinitrobenzene sulphonic acid method; $P < 0.05$), probably due to differences in pH values that impacted on the residual chymosin activity. Texture profile analysis showed that RF were firmer than FF ($P < 0.05$); however, an increase in LS led to cheeses with increased hardness, springiness and cohesiveness. Descriptive sensory analysis showed that RF were more translucent, firmer and more astringent than FF ($P < 0.05$), whereas later LS led to cheeses with decreased acid perception. This study suggests that the quality characteristics of cheeses can be affected by a reduction in fat content. However, standardization of cheese manufacture protocols will also have to consider control of acid development to achieve desired levels of acidity in cheeses made from milks from late LS.

Key Words: reduced-fat goat cheese, late lactation, cheese ripening

303 Comparison of curd washing and lactose standardization for manufacture of Colby cheese.

H. Jiang*, S. Govindasamy-Lucey, J. Jaeggi, M. Johnson, and J. A. Lucey, *Center for Dairy Research, Madison, WI.*

Curd washing (CW), a technique which adds water to curds to aid in lactose removal, is mostly used in the manufacture of Colby cheese. However, CW uses a lot of water and if improperly done, curds may still contain excessive lactose which eventually results in acidic Colby. Lactose-standardization (LS) is a technique that was developed by our center to remove some lactose from milk through ultrafiltration and diafiltration and thus eliminate the need for CW. In our study, the efficiency/feasibility of lactose removal through CW or LS technique were compared on Colby cheese manufacture (n = 5). CW-milk was regular cheese milk with ~4.3% lactose, while LS-milk was standardized to 2.2% lactose, but had similar casein (~2.5%) and casein-to-fat ratio (~0.7) as CW-milk. CW-milk and LS-milk were pre-acidified to reduce the insoluble colloidal calcium phosphate. After the whey was completely drained, CW curds were washed with 32°C water at a ratio of 1:14 to milk (a typical CW level used in industry) for 10 min. There was no curd washing step used for LS curds. CW and LS cheeses had similar moisture, fat, protein, salt, total calcium content and proteolysis (soluble nitrogen as a percentage of total nitrogen in both pH 4.6 and 12% TCA tests at 14 d). LS cheeses had higher pH ($P < 0.05$) and less lactic acid content than CW cheeses during the 6 mo-ripening period. LS cheeses had ~40% lower hardness values (measured by texture analyzer, $P < 0.05$) than CW cheeses at 60 d of ripening but both had similar melting points (50°C, measured by dynamic small amplitude oscillatory). Changes in sensory flavor and texture properties were evaluated by trained sensory panelists (8–10) using quantitative descriptive analysis. Most of the sensory characteristics were similar ($P > 0.05$) between CW and LS cheeses, except that LS cheeses had lower particle size, acid and astringency values ($P < 0.05$). Thus, LS technique has great potential in replacing CW technique used in traditional Colby cheese manufacture to control the amount of lactose and thus acidity in cheese.

Key Words: Colby, curd washing, lactose standardization

304 Effect of processing variables on viscoelastic properties and textural attributes of heat-acid coagulated milk product paneer.

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Paneer, a popular unripened soft cheese of India, is prepared by heat and acid coagulation of milk. The study investigated the effect of milk heating (90°C/no hold and 90°C/10 min) and coagulation temperature (70, 80 and 90°C) on structure, texture and rheological properties of paneer. Paneer structure was observed using confocal laser scanning microscopy. Textural attributes were determined using a texture analyzer. Viscoelastic properties i.e., storage modulus (G’) and loss modulus (G”) were measured using a frequency sweep test. Transient rheological responses of paneer under constant stress (50 Pa) and strain (1%) were measured using creep compliance and stress relaxation tests, respectively. Moisture content decreased in paneer samples with increasing milk coagulation temperature (~50.8% and ~45% wt/wt at 70°C and at 90°C coagulation, respectively). Hardness, chewiness and storage modulus (G’) values of paneer samples increased with an increase in coagulation temperature. Paneer, coagulated at 90°C, exhibited highest hardness (4.24 ± 0.05 g; heat treatment to 90°C/no-hold) and chewiness (1.65 ± 0.09 g; heat treatment 90°C/10 min) values when compared with other treatments. However, milk heating conditions had no significant ($P < 0.05$) effect on creep parameters indicating better structure retention in samples coagulated at 90°C (lower deformation rate) and weak structure in samples coagulated at 70°C (higher deformation rate). Stress relaxation was also slower when paneer prepared at lower coagulation temperatures indicating that increase in coagulation temperature from 70 to 90°C resulted in more elastic paneer. Confocal laser scanning microscopy revealed that increasing coagulation temperature resulted in samples with a more compact and dense protein matrix. Higher total solids content and lower moisture content of paneer samples were associated with firmer texture and denser protein structure which could be attributed to increased coagulation temperatures. The present study offers insight into the effects of processing conditions on the intricate relationships between structure-rheology-texture and functionality of paneer.

Key Words: structure, rheology, paneer

Production, Management, and the Environment

305 Estrus prediction of cows and heifers with an activity and rumination monitoring system in an organic grazing and a low-input conventional dairy herd. B. J. Heins* and K. Minegishi, *University of Minnesota*, *St. Paul, MN*.

The objective of this study was to evaluate estrus detection with an activity and rumination system (ARS) in a seasonal calving organic grazing (ORG) and a low-input conventional (CONV) dairy herd. Data provided by the ARS was used with machine learning techniques to create estrus prediction models for heifers and cows. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN from January 2016 to August 2019. Cows calve seasonally on this research farm. Cows that calved in the spring were bred during the summer and cows that calved in the autumn were bred during the winter. The study had 4 winter breeding seasons (December to February) and 4 summer breeding seasons (June to August). During each breeding season, activity and rumination were monitored electronically using an ear-tag accelerometer sensor (CowManager SensOor, Agis Automatisering BV, Harmelen, the Netherlands). Estrus alerts of individual cows provided by the activity and rumination monitoring system were used to determine agreement with the breeding date of a cow. The study included 1,671 breeding dates from 917 cows and 180 breeding dates from 126 heifers (HEF). The estrus prediction analysis focused on 8 machine learning algorithms with R statistical version 3.51 (R Foundation, Vienna, Austria). Model prediction was assessed by receiver operation characteristic (ROC) curves. For the winter breeding season, the ROC curves had 80% sensitivity (SN) with 97% specificity (SP). The ROC curves for the summer breeding season were lower (72 to 77% SN with fixed 97% SP) for cows in CONV herd, for cows in ORG herd (50% SN), and for HEF (63 to 71% SN). With a lower sensitivity and a higher positive predictive value (PPV), summer prediction results were 76 to 81% SN and 54 to 70% PPV for CONV, 52 to 64% SN and 42 to 50% PPV for ORG, and 69 to 76% SN with 51 to 68% PPV for HEF. The custom models developed using the raw ARS data showed the potential range of sensitivity and specificity that can be achieved with these data.

Key Words: automated estrus detection, grazing, low-input dairy

306 Revealing the effects of reproduction and turnover rate on farm profitability through herd structure dynamics. W. Li* and V. E. Cabrera, *University of Wisconsin-Madison*, *Madison, WI*.

Dairy herd structure influenced by reproduction and turnover rate determines the whole farm profitability because each group of animals has a distinctive production efficiency reflected in costs and revenues. The objective of this study was to reveal the interactions among reproductive performance, turnover rate, herd structure, and farm profitability. A monthly Markov chain model mimicking cows and youngstock was used after performance evaluation with a 7-mo farm data set. A simulated herd with 45% turnover rate (TR), 16% 21-d pregnancy rate (21-d PR), and 0% voluntary calf culling rate (VCC) was used as the base. The base herd was then imposed 25% TR or 40% 21-d PR and combinations of VCC. Results showed that decreased TR (45 to 25%) increased the percentage of multiparous cows (50.0 to 58.4%); cow longevity (49.0 to 58.2 mo); net return (NR, +$4/cow per mo); and net return after other costs (NRA, management included, $2 /cow per mo). Greater 21-d PR (16 to 40%) increased the percentage of pregnant cows (49.6 to 67.3%); percentage of multiparous cows (50.0 to 65.5%); and the ratio of heifers to adults (0.80 to 1.08). Greater 21-d PR increased the percentage of cows less than 150 DIM (43.4 to 47.9%). Greater 21-d PR also increased NR by $7/cow per mo but decreased NRA by $3 /cow per mo due to greater replacement rearing costs. Using an optimization algorithm, we maximized NRA at breakeven on-farm replacement supply and demand at 44% VCC when 25% TR and 40% 21-d PR. Maximum NR and NRA were then $10 and $18 /cow per mo, respectively, greater than the base. To alleviate the volatility of NR and NRA in the short term, managerial changes could be introduced progressively, still reaching same herd performance and economic outcomes in the long term. Overall, and under current market conditions, farms with better reproduction performance, lower turnover rate, and raising only the required number of replacements, are more profitable.

Key Words: reproduction, turnover rate, herd structure

307 Trends in the use of beef semen in dairy herds in the western United States. J. M. V. Pereira*[1,2], M. I. Marcondes[1], and F. C. Ferreira[2], [1]*Universidade Federal de Vicosa*, *Vicosa, MG, Brazil*, [2]*Department of Population Health and Reproduction, University of CaliforniaDavis, CA*.

Beef semen has been used as a strategy in the dairy industry to improve herd profitability. Our objective was to describe the trends in the use of beef semen in dairy herds in the western US. DHIA data from 2010 to 2017 was obtained from AgriTech. We used SAS 9.4 for data management and descriptive statistics. Cows with more than 10 lactations were excluded from our data set (498 cows, 2,083 lactations). Our final data set contained data from 460 herds (CA:380, ID:29, OR:22, NM:19, WA:10), 2,611,805 cows, 5,510,083 lactations, and 12,515,585 breedings. Cows were classified according to their parity order (1st, 2nd, and 3rd and plus), milk production: MP, (low (<34.3 Kg/d, P25), medium (34.3 - 43.5 kg/d) and high (>43.5 Kg/d, P75)), and breed (Holstein HO and Jersey JE). Herds were grouped by: herd size (small: < 694 cows (n = 115, P25), medium: 694 - 2,296 (n = 230) and large: > 2,296 (n = 115, P75)), herd type (HO >70% Holstein cows, (n = 373, 81% of all herds), JE >70% Jersey cows, (n = 77, 17% of all herds) and mixed (n = 10, 2% of all herds)), and conception rate: CR, (low: < 26.1% (n = 134, P25), medium: 26.1 - 32.4% (n = 199) and high: > 32.4% (n = 127, P75)). Beef semen was used in 152 herds (33% of all herds). In 2014, 0.2% of all breedings were beef (3,341 out of 1,692,420). In 2017, beef breedings represented 1.3% of all breedings (20,064 out of 1,596,485), an increase of 600%. By parity order, 45% of all beef semen breedings were on 3rd and plus lactation. Medium MP cows used BS in 0.8%, while low and high MP cows used BS in 0.7% of breedings from 2014 to 2017. In 2017, the percentage of beef semen breedings was 1.4%, 1.2% and 0.6% in large, medium and small herds. Holstein herds had 98% of all beef semen breedings. For CR, 1.7% represents the use of beef semen in low, 1.3% in medium and 0.9% in high CR herds in 2017. From 2014 to 2017, sexed dairy semen breedings increased 300%, from 34,567 in 2014 to 106,383 breedings in 2017. In conclusion, the increase in beef semen use has been led by dairies using large Holstein herds, with medium production level, low CR, and greater parity.

Key Words: trends, beef semen in dairy cattle, reproductive management

308 Beef semen management practices in California dairy herds. J. M. V. Pereira*[1,2], D. Bruno[3], M. I. Marcondes[1], and F. C. Ferreira[2], [1]*Universidade Federal de Vicosa*, *Vicosa, MG, Brazil*, [2]*Department of Population Health and Reproduction, University of California*, *Davis, CA*, [3]*University of California Cooperative Extension*, *Fresno, CA*.

Beef semen (BS) usage in dairy cows has been employed as a reproductive management strategy. Our aim was to describe the CA dairy farmer's management practices on the use of BS. On Jan 17th, 2020 we mailed a 1,070 BS surveys to dairy producers in CA. Survey questions (n = 43) were divided into dairy information (n = 10), BS (n = 17), and sexed semen (SS, n = 6) usage. As of Feb 14th, 2020, 100 dairies (9.3%) representing 8.6% of all cows in CA (n = 149,456) had returned the survey. This abstract will present our preliminary results. Herd size averaged 1,590 milking cows (range 105–5,500) with 34.8 kg of milk/cow/d (range 18–43). Of the 100 respondents, 6% did not identify the herd breed, 71% were Holstein, 13% mixed (Holstein and Jersey), 8% Jersey and 2% crossbred. Most producers are using BS (n = 77, 77%), of which 64.9% are using Angus, followed by Wagyu (11.7%) and Limousin (9.1%). Preference of the calf ranch (42.7%), cost (40%) and calving ease (24%) were the main factors driving the selection of the BS. The majority of the respondents (70%) started BS use less than 3 years ago, and 28.9% of all dairies are breeding more than 30% of all eligible cows with BS. Dairies are considering cow's reproductive performance (73.7%), lactation number (46.1%), milk production (40.8%) and genomic testing (35.5%) as their criteria to select them to receive BS. Regarding lactation number, the majority of herds (59.2%) start breeding with BS on 3rd lactation and plus followed by 34.7% on 2nd, 30.6% on heifers, and 11.6% on 1st. Concerning reproductive performance, 45.3% start breeding after 3rd breeding, 32.8% after 4th, and 28.1% after 5th or more. Extra profit and managing the extra number of heifers were the mains reasons for using BS. Among the BS users, 63% also use SS. Lactation number (45.2%) and genomic testing (42.5%) are the main factors to select cows for SS breeding. When asked about their perspectives for the crossbred future market, 58.1% of the respondents using BS agreed that the crossbred calf price will decrease. Survey results demonstrated the widespread use of beef semen in dairies in CA and the main breeding strategies adopted by dairy farmers.

Key Words: beef semen, dairy management, California

309 An application of the Ruminant Farm System Model (RuFaS): The use of a combination of sexed and beef semen on dairy herds. M. Li*[1], V. E. Cabrera[1], and K. F. Reed[2], [1]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI*, [2]*Department of Animal Science, Cornell University, Ithaca, NY.*

A whole-farm integrated simulation model, the Ruminant Farm System model (RuFaS), is being collaboratively developed by multiple institutions to study dairy farm sustainability and profitability comprehensively. RuFaS is coded in Python and has a modularized framework of key farm components like animal, manure, soil and crop, and feed storage. In the animal module, a Monte Carlo stochastic model of the animal simulates individual animals and the overall herd. One application of the animal life cycle model was to evaluate the economic impact of a recent trend in reproductive management: breeding cows with a combination of sexed and beef semen. Our objective is to compare 2 reproductive management scenarios using the RuFaS animal life cycle model: (1) using conventional semen for all cows, (2) using 40%, and 15% sexed semen in the 1st and 2nd lactation cows, and beef semen in all other cows. A 1,000-cow herd following a Double-Ovsynch protocol was simulated, herd size was maintained with the heifers kept on-farm for both scenarios, and extra calves are sold as Holstein or beef calves. We set the conception rate (CR) for the first insemination of 1st lactation cows to 55% for beef and conventional semen. Then, we decreased the CR by 5% and 10% for 2nd and later lactations. Each additional insemination after the first had a 2.6% lower CR. We assigned sexed semen to be 80% CR of beef semen. Estimates of yearly net return (NR) included income from milk, sold calves value, slaughter value and costs from the feed, farm operations, and reproduction management, which included semen, AI, pregnancy checks. Results showed an NR of $2,237/cow per year for sexed and beef semen scenario, higher than the NR of $2,097/cow per year for conventional semen scenario. Once the animal life cycle model is fully connected to nutrition and farm management models in the animal and/or other modules, it will be able to provide complete farm profit analyses and multiple possibilities for scenario testing.

Key Words: whole-farm simulation, beef semen, RuFaS

310 Daily vaginal temperature in Girolando cows from three different genetic compositions under natural heat stress. L. d. R. Carvalheira*[1], R. R. Wenceslau[1], L. d. S. Ribeiro[2], B. C. d. Carvalho[3], Á. M. Borges[1], and L. S. d. A. Camargo[3], [1]*Departamento de Clínica e Cirurgia Veterinárias, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*, [2]*Departamento de Patologia e Clínica Veterinária, Faculdade de Veterinária, Universidade Federal Fluminense, Niterói, RJ, Brazil*, [3]*Laboratório de Reprodução Animal, Embrapa Gado de Leite, Juiz de Fora, MG, Brazil.*

The present trial evaluated the effect of proportion of Holstein genetic on crossbred composition (H) and temperature-humidity index (THI) on vaginal temperature (VT) of Girolando dairy cows maintained under tropical pasture during warm seasons. Vaginal temperature was monitored from 615 Holstein x Gir cows (1/2 H = 284, 3/4 H = 248 and 7/8 H = 83) from 6 Brazilian farms from January to March of 2016 and 2017. VT of each cow at each hour of the day and the respective THI were averaged per hour across all monitoring days to generate an averaged value for VT and THI during 24h. A linear mixed model with repeated measures using REML method for (co)variance components estimation procedure was employed. The final model adjusted the VT for the effects of farm, year, pregnancy status, body condition score and milk yield. Fixed effects were evaluated by ANOVA and tested with Tukey test in R 3.6.1 software (R Core Team, 2019). Overall mean of VT, air temperature (AT) and THI were, respectively, 39.06 ± 0.52°C, 25.63 ± 0.40°C and 75.06 ± 3.96. VT had moderate positive correlation with THI ($r^2 = 0.45$, $P < 0.001$) and AT ($r^2 = 0.46$, $P < 0.001$). The VT had estimated linear increase of 0.05 degrees of each THI unit increase ($P < 0.001$). The daily average VT was affected by the proportion of Holstein (H) on genetic composition (1/2 H = 38.95 ± 0.06 a, 3/4 H = 39.08 ± 0.06 b and 7/8 H = 39.09 ± 0.06 b; $P < 0.001$). The difference of VT among the 3 crossbred groups varied in function of the time of the day, from 12:00 to 20:00 h ($P < 0.001$). 3/4 H and 7/8 H cows had similar VT and higher than 1/2 H cows during all this period. In conclusion, Girolando cows are subjected to heat stress in tropical condition during warm seasons and also, Holstein proportion higher than 3/4 reduces its thermoregulatory efficiency. Strategies to alleviate heat stress should be use in dairy farms that raise Girolando cows in tropical conditions.

Key Words: core body temperature, crossbreed, tropical environment

417 Automated real-time integration of data from multiple sensors and nonsensor systems for prediction of dairy cow and herd status and performance. M. M. Pérez*[1], G. Rubambiza[2], B. Barker[3], H. Weatherspoon[2], and J. O. Giordano[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY*, [2]*Department of Computer Science, Cornell University, Ithaca, NY*, [3]*Cornell Center for Advanced Computing, Cornell University, Ithaca, NY.*

Our goal was to build a software infrastructure to integrate data from multiple heterogeneous cow wearable and non-wearable sensors, herd management software, and climate sensors for subsequent data analytics. To address variation in exposed interfaces and data update frequencies to our aggregator, a Python client was prototyped to periodically check for unprocessed files stored on a farm PC. The unprocessed files are forwarded to a cloud-based aggregator. Files are structured to be processed into Google's Protocol Buffer (protobuf) objects. Protobufs allow for minimalistic encoding/decoding of data limiting the size of probable sparse messages such as cow health status and sensor data. Protobufs read and modularize the structures of Excel (XLSX and CSV) files into a singular structure used by the aggregator. In preliminary experiments using protobufs, our client achieved nearly an order of magnitude reduction in the size of data transferred to the aggregator (e.g., 14KB xlsx file to 1500 byte protobuf message). To streamline communication between the data aggregator and analytics services for prediction with improved modularity and reduced data sparsity, incoming data is stored into a NoSQL Cassandra database with a distinct table for each data source. This framework allows querying data using a cow number and timestamp. To transfer data to an analytics pipeline, RESTful queries are implemented to the data aggregator, which serves up CSV files generated from the database. Thus far, the data aggregator receives and integrates data from the herd management software, in-line milk sensors (yield and components), walk-in scale, a physical activity and resting behavior leg-mounted sensor, a rumination and eating behavior neck-mounted sensor, a reticulo-rumen temperature sensor, and climate conditions sensors in and outside barns. In summary, we built a software system that automatically integrates in real-time heterogeneous data from diverse sources at a dairy farm. Aggregated data is transferred to data analytic tools for prediction of cow and herd status and performance.

Key Words: data integration, sensor, prediction

Ruminant Nutrition General

311 Gastrointestinal morphology of preweaned dairy calves fed whole milk powder or a high-fat milk replacer. S. C. Mellors*[1], A. C. Welboren[1], J. Wilms[2], L. N. Leal[2], J. Martín-Tereso[2], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph*, *Guelph, ON, Canada*, [2]*Trouw Nutrition Research and Development*, *Amersfoort, the Netherlands*.

The objective of this study was to evaluate effects of feeding a milk replacer (MR) of similar macronutrient composition to bovine whole milk and whole milk powder on growth, health and gastrointestinal (GIT) morphology. Male Holstein calves (n = 18, 1–3 d of age) were individually housed and blocked by day of arrival and age. Calves were not offered any solids to control nutrient intake and were fed 3L (135g/L) 3 times daily of either: 1) whole milk powder (WM, 26.0% fat, 24.5% protein, 38.0% lactose, n = 9); or 2) high fat MR (HF, 25% fat, 22.5% protein, 38.1% lactose, n = 9). BW (BW) was measured weekly and feed intake was recorded daily. Calves were euthanized at d 28 to obtain organ weights and intestinal samples to assess GIT morphology via histological analysis. Data were analyzed in SAS software using repeated measures GLIMMIX for weekly measures and Proc MIXED was used for dissection data. Weekly intake of MR (WM = 8.5L vs, HF = 8.6L; SE = 0.16L; $P = 0.3$) and BW at the time of dissection (WM = 56kg vs HF = 55kg; SE = 1.8; $P = 0.8$) did not differ between treatments throughout the experiment. Similarly, feed efficiency and metabolizable energy (ME) intake by week did not differ between treatment groups. In foregut digestive compartments, rumen (498g vs. 365g; SE = 39.6; $P = 0.02$), reticulum (100g vs. 73g; SE = 7.9g; $P = 0.04$), and omasum (215g vs. 148g; SE = 17.6g; $P = 0.02$), weights were found to be greater in WM than HF, respectively. Weights of the duodenum and ileum were similar between treatment groups but jejunum weight was greater in WM compared with HF (1462g vs. 1172g; SE = 63.7g; $P = < 0.0001$). Surface area of the duodenum and ileum did not vary between treatment groups; however, surface area of distal jejunum trended to be greater in WM than HF (1.1 vs. 0.99; SE = 0.08; $P = 0.1$). In visceral organs, no differences were found, albeit spleen weight was higher in HF than WM (270g vs. 2737g; SE = 10.2g; $P = 0.01$). Overall, the results suggest that the composition, other than macronutrient composition, of the liquid diet may influence GIT morphology during the preweaning period without affecting growth or feed efficiency.

Key Words: growth, histology, macronutrient

312 Effect of residual feed intake on nutrient digestion and milk production of lactating Holstein cows fed high and low starch diets. X. Dai* and K. F. Kalscheur, *U.S. Dairy Forage Research Center, USDA-ARS*, *Madison, WI*.

The objective of this study was to evaluate whether the difference in nutrient digestion and milk production could be explained by residual feed intake (RFI) in lactating cows. A total of 62 lactating Holstein cows (137 ± 3.8 DIM) were fed either high starch (HS; 27% starch, 29% NDF) or low starch (LS; 13% starch, 37% NDF) diets. The crossover designed experiment consisted of 2 56-d treatment periods with 11-d diet adaptation. Residual feed intake (RFI) is defined as the difference between observed and expected metabolizable energy intake (MEI). High RFI cows ate more than expected and were less efficient. Cows were classified as 1 of the following: highest 10% RFI (HRFI), lowest 10% (LFRI) and medium RFI (MFRI; the remaining 80%). Greater DM and OM total-tract digestibility ($P < 0.01$), but lesser NDF and ADF total-tract digestibility ($P < 0.01$) was observed in cows fed HS diet compared with LS diet. Greater molar proportion of propionate ($P < 0.01$), valerate ($P < 0.01$) and isovalerate ($P = 0.05$) were found in HS-fed cows compared with LS-fed cows. Greater DMI ($P < 0.01$), MEI ($P < 0.01$), and milk yield ($P < 0.01$) were observed in HS-fed cows compared with LS-fed cows. Cows fed the HS diet resulted in greater milk protein concentration and lesser milk fat concentration compared with cows fed the LS diet. LRFI cows consumed 5 kg less DMI but produced 5 kg more milk compared with HRFI cows fed the HS diet. However, when cows were fed the LS diet, the LRFI cows had 6 kg less DMI and produced 5 kg less of milk compared with HRFI cows. Meanwhile, LRFI cows had more BW loss when fed the HS diet but more BW gains when fed the LS diet. Decreased total-tract digestibility and lower total VFA production resulted when LRFI cows were fed the HS diet compared with HRFI cows fed the HS diet. Our results suggest that the difference in nutrient digestion and milk production across different RFI groups are dependent on dietary starch levels. Dietary impacts need to be considered when using RFI as a feed efficiency selection tool for lactating cows.

Key Words: residual feed intake, starch, cow

313 The effects of nutritional management in early lactation and dairy cow genotype on milk production and metabolic status. E. L. Brady*[1], M. B. Lynch[2], K. M. Pierce[2], A. G. Fahey[2], and F. J. Mulligan[1], [1]*School of Veterinary Medicine, University College Dublin*, *Belfield, Dublin, Ireland*, [2]*School of Agriculture and Food Science, University College Dublin*, *Belfield, Dublin, Ireland*.

High levels of milk production coupled with low feed intake causes negative energy balance in early lactation, especially in the first month post calving. Therefore, specific nutritional management at this time may improve nutritional and metabolic status with the possibility of genotypes responding differently. Thus, the objective of this study was to compare the effects of contrasting nutritional management strategies and dairy cow genotypes on milk production and metabolic status during early lactation for grazing cows. Sixty Holstein-Friesian cows were blocked on calving date, previous 305-d milk yield, BCS and genotype. Cows of high fertility low milk (HFLM) and low fertility high milk (LFHM) genotype (based on the 2019 Economic Breeding Index evaluation, ICBF Ireland) were randomly assigned to 1 of 2 treatments in a 2 × 2 factorial, randomized complete block design. The dietary treatments were (1) allocation of 21 kg of DM of a tailored TMR (TMR, n = 30); (2) ad libitum access to fresh pasture plus an allowance of 3 kg of concentrates (G, n = 30). These diets were offered for the first 30 DIM. Post 30 DIM, TMR cows joined the G treatment and were managed similarly until 100 DIM. Blood samples were taken weekly in the first month after calving and BCS recorded every 2 wk. BCS and BCS loss were analyzed using PROC MIXED of SAS (9.4; 2012) and blood metabolites using repeated measure MIXED procedure of SAS (9.4; 2012). Feeding TMR for the first 30 DIM significantly improved the metabolic status as NEFA (−0.12 mmol/L, $P < 0.001$) and BHB (−0.10 mmol/L, $P < 0.001$) were lower compared with the G treatment. Overall, there was no significant differences in BCS when comparing TMR (2.92) with G (2.88, $P = 0.49$) cows. However, TMR cows had a lower BCS loss (−0.23) from calving until 60 DIM compared with G cows (−0.41, $P < 0.01$). Genotype did not have an influence on metabolic status. In conclusion, specific NM in the first month after calving improves metabolic status and significantly reduces BCS loss up to 60 DIM regardless of genotype.

Key Words: early lactation, nutritional management, genotype

314 Feeding behavior of lactating dairy cows fed switchgrass (*Panicum virgatum*) as a replacement for wheat straw in a total mixed ration. R. L. Nagle*[1], B. R. Lemay[1], M. Thimmanagari[2], T. J. DeVries[1], and A. J. Carpenter[3], [1]*Department of Animal Biosciences, University of Guelph*, *Guelph, ON, Canada*, [2]*Ontario Ministry of Agriculture, Food and Rural Affairs*, *Guelph, ON, Canada*, [3]*CSA Animal Nutrition*, *Dayton, OH*.

The objective of this study was to evaluate the feeding behavior of lactating dairy cows fed switchgrass (*Panicum virgatum*; SG) as a replacement for wheat straw (WS) in TMR. Two experiments (Exp) were performed where SG replaced WS in TMR for lactating Holstein cattle (n = 24; parity

= 2 ± 1.2; DIM = 162 ± 19.3 and 215 ± 21.3, for Exp 1 and 2, respectively). Each Exp consisted of 2 periods in a crossover design, with 11d of adaptation and 10d of sample collection. In Exp 1, either WS or SG were fed in the TMR at 1.8% of diet DM (starch = 22.1 ± 1.74% or 20.6 ± 2.02% of diet DM, respectively). Cows were fed higher starch rations in Exp 2, with either WS or SG in the TMR at 3.6% of diet DM (starch = 29.6 ± 1.54% or 27.6 ± 2.45% of diet DM, respectively). Intake was monitored with automated feeders to determine DMI (kg/d), time spent feeding (min/d), total meal time (min/d), meal length (min/meal), meal size (kg/meal), feeding rate (kg/min), meal frequency (meals/d), and the interval between meals (IMI; min/d). Data were analyzed using a mixed-effect linear regression model, with day as a repeated measure. Two cows were excluded from analysis in Exp 1 for health and stealing feed, and 2 cows were excluded from Exp 2 for stealing. In Exp 1, DMI was greater for cows fed SG (25.1 ± 0.69 vs. 24.22 ± 0.69 kg/d; $P \leq 0.01$) as was meal frequency (8.8 ± 0.36 vs. 8.1 ± 0.36; $P \leq 0.01$), but there was no effect on meal size ($P = 0.11$). Cows on the SG diet had a shorter meal length (37.3 ± 2.06 vs. 39.6 ± 2.05 min/meal; $P = 0.02$) and IMI (145.5 ± 6.80 vs. 163.1 ± 6.69 min/d; $P \leq 0.01$). Feeding rate, time spent feeding, and total meal time were not affected ($P \geq 0.14$). In Exp 2, DMI (25.0 ± 0.65 vs. 25.6 ± 0.65 kg/d; $P = 0.02$) and feeding rate (0.135 ± 0.0086 vs. 0.143 ± 0.0086 kg/min; $P \leq 0.01$) were lesser for cows fed SG. Time spent feeding for cows fed SG was greater (208.3 ± 9.11 vs. 201.7 ± 9.13 min/d; $P = 0.01$), but meal frequency, size, length, total meal time, and IMI were not affected ($P \geq 0.56$). In conclusion, feeding behavior was affected by inclusion of SG in TMR, however, the response varied depending on dietary starch level and inclusion of SG.

Key Words: switchgrass, feeding behavior, starch

Ruminant Nutrition: Protein/Amino Acids

315 Using plasma sulfur amino acid concentrations to differentiate the metabolizable methionine contributions of rumen-protected methionine products. N. L. Whitehouse*, A. R. Blanchard, S. M. Hollister, and L. H. P. Silva, *University of New Hampshire*, *Durham, NH*.

The objective was to investigate if the plasma AA dose-response method, using total sulfur AA (TSAA) concentrations as a % of TAA – SAA concentrations, could statistically differentiate the metabolizable Met content in 4 unique RP-Met products in lactating Holstein cows. Ten multiparous Holstein cows (175 ± 59 DIM) were used in a replicated 5 × 5 Latin square with 7-d experimental periods. The 5 treatments were: 1) a negative control (CON); 2) 24 g/d Met from Smartamine M (SMM; Adisseo; Met bioavailability ≥80%); 3) 24 g/d Met from AminoShure-XM (AXM; Balchem); 4) 24 g/d Met from MetiPEARL (MPL; Kemin); and 5) 24 g/d Met from Timet (TMT; Vetagro). Intake and milk yield were measured daily. Blood was collected from the tail vein the last 3 d per period at 2, 4, 6 and 8 h after the 0500h feeding. Data were analyzed using MIXED and REG procedures of SAS. Significance was declared at $P \leq 0.05$. Milk yield (37.1 kg/d) and DMI (25.6 kg/d) were not affected by treatment. Plasma Met, cystathionine/allocystathionine, and TSAA concentrations were increased for cows fed AXM compared with CON and MPL, and further increased for SMM compared with AXM (Table 1; $P < 0.001$). Based on comparison of dose-response slopes using TSAA expressed as a percentage of TAA-TSAA, AXM, MPL, and TMT were 35.0 (±4.0), 9.3 (±3.7), and 24.0% (±7.2) as effective as SMM in providing metabolizable Met.

Key Words: plasma, sulfur amino acids, bioavailability

316 In situ rumen degradability and intestinal digestibility of rumen-protected lysine products. F. Francia[1], C. Faturi[2], M. E. Rodriguez-Prado[1], and S. Calsamiglia*[1], [1]*Animal Nutrition and Welfare Service, Univeristat Autonoma de Barcelona*, *Bellaterra, Spain*, [2]*Universidad Federal Rural de la Amazonia*, *Belem, Brazil*.

The objective of this study was to determine and compare rumen degradation and in vitro intestinal digestibility of 3 ruminally protected (RP) lysine products: LysiGEM and LysiPEARL (Kemin Animal Nutrition and Health); and AjiPro (Ajinomoto). Ruminal degradability and intestinal digestibility of different RP-Lys sources were studied using the in situ nylon bag technique and the modified 3-step in vitro technique, respectively. The RP-Lys products consisted of Lys coated with rumen-stable compounds. Samples (0.38 ± 0.01 g) of each RP product were weighed into nylon bags and incubated in the rumen for 0, 2, 4, 8, 16, 24 and 48 h in duplicate bags for each incubation time in 2 consecutive experimental periods. An empty bag was also included as a correction factor for each sample at each incubation time. Ruminal degradability was calculated with a simple exponential function ($Y = a$+b*(1-exp(-ct))), where a was the amount of N disappearing from the bag at 0 h; b was the potentially degradable fraction; and c was the degradation rate of the potentially degradable fraction. The effective degradability of N (EDN) was calculated as: EDN (%) = $a+[(b*c)/(c+k)]$; where k (0.10/h) is the estimated rate of outflow from the rumen, and a, b, and c are the same as described earlier. Treatment effects adjusted by period were analyzed using the GLM of SAS (Cary, NC) and significance declared at $P < 0.05$. Solubility (%) was higher in LysiGEM (20.9) and LysiPEARL (26.8) compared with AjiPro (2.6). Rate of degradation (/h) was higher in LysiPEARL (0.048) compared with AjiPro (0.015) and LysiGEM (0.013). Effective degradation (%) was highest in LysiPEARL (49.2), intermediate in LysiGEM (21.4) and lowest in AjiPro (9.2). However, intestinal digestibility (%) was lowest in AjiPro (47.9) compared with LysiGEM (87.3) and LisiPEARL (95.3). Bioavailability (%) was highest in LysiGEM (63), intermediate in LysiPEARL (48.7) and lowest in AjiPro (44.5). There are relevant differences in ruminal degradability and intestinal digestibility of Lysine among different RP-Lys that have an important impact on overall bioavailability.

Key Words: protected lysine, ruminal degradability, intestinal digestibility

317 Lactation performance and nitrogen utilization of dairy cows on diets including unfermented or fermented yellow wine lees mix. K. Y. Yao[1], Z. H. Wei[1], Y. Y. Xie[1], D. M. Wang*[1], H. Y. Liu[1], M. R. Ma[2], and J. X. Liu[1], [1]*Institute of Dairy Science, College of Animal Sciences, Zhejiang University*, *Hangzhou, China*, [2]*College of Agriculture and Bioengineering, Jinhua Polytechnic*, *Jinhua, China*.

Yellow wine lees (YWL) consist of a high content of CP (about 25%) with relatively low price, and the CP quality may be improved by microbial fermentation. This study was conducted to investigate lactation performance and nitrogen (N) utilization in dairy cows receiving unfermented and fermented YWL mix as alternative protein source for soybean meal. Fifteen multiparous mid-lactation Chinese Holstein dairy cows with similar parity (2.30, SD 0.32), DIM (190, SD 15.2) and milk yield (25.0, SD 0.45 kg) were used in a replicated 3 × 3 Latin square design. The designed TMR contained: (1) 18% soybean meal (Control), (2) 11% unfermented YWL mix (UM), and (3) 11% fermented YWL mix (FM), respectively. Diets were isonitrogenous and isocaloric, with a forage-to-concentrate ra-

Table 1 (Abstr. 315). Plasma AA concentrations, dose-response slopes, and calculated concentrations of metabolizable Met in RP-Met supplements

Item	CON	AXM	MPL	SMM	TMT	SEM	*P*-value
Methionine, µ*M*	24.6^{d}	29.1^{b}	25.2^{cd}	43.6^{a}	27.4^{bc}	0.87	<0.001
Cystine, µ*M*	20.1	20.7	20.0	21.2	20.0	0.36	0.10
Cystathionine/Allocystathionine, µ*M*	1.81^{c}	2.02^{b}	1.84^{c}	2.61^{a}	1.98^{bc}	0.08	<0.001
Homocystine, µ*M*	2.54	2.41	2.60	2.58	2.43	0.26	0.98
Taurine, µ*M*	46.2	48.2	42.5	52.5	45.7	3.68	0.42
TSAA, µ*M*	95.4^{c}	102.5^{b}	92.3^{c}	122.6^{a}	97.7^{bc}	2.71	<0.001
Slope TSAA (%TAA − TSAA)	—	0.0192^{b}	0.005^{c}	0.0547^{a}	0.131^{b}	0.002	<0.001
Metabolizable Met, g/kg[1]	—	202	42	636	108	—	—

[a–d]Means within rows differ at $P < 0.05$.

[1]Analyzed Met content of products: AXM 72.3%, MPL 56.0%, SMM 79.5%, and TMT 56.3%.

tio of 60:40. Each period lasted for 20 d, with the first 15 d for adaptation. Contents (%DM) of CP, NDF, and ADF were 26.3, 36.4, and 15.6; and 31.3, 41.1, and 19.0 for unfermented and fermented YWL, respectively. Data on lactation performance and N utilization taken over 5 d measurements were analyzed using the PROC MIXED procedure with repeated measurement in SAS software. The DM intake ($P = 0.04$), milk yield ($P = 0.02$), and milk protein yield ($P = 0.02$) were higher in cows fed the control and FM diets than in the UM-fed cows (Table 1). Milk composition, feed efficiency, and N efficiency were not different ($P > 0.05$) among the 3 groups. The cows fed the UM diet showed lower microbial protein ($P = 0.04$) and metabolizable protein ($P < 0.01$) than the cows fed the control or FM diets. Feeding of the FM-fed cows tended to have greater income over feed cost than the control ($P = 0.06$). In summary, both unfermented and fermented YWL can be used as a protein source in the diets for lactating cows and inclusion of the fermented YWL had no adverse effects on lactation performance and N utilization but with greater profit.

Table 1 (Abstr. 317). Feed intake and lactation performance in dairy cows

Item	Control	UM	FM	SEM	*P*-value
DM intake, kg/d	19.4^{a}	18.1^{b}	19.5^{a}	0.43	0.04
Milk yield, kg/d	21.5^{a}	23.2^{b}	24.3^{a}	0.39	002
Milk protein yield, g/d	846^{a}	814^{a}	855^{a}	13.4	0.02
Milk composition, %					
Fat	4.50	4.36	4.34	0.12	0.57
Protein	3.45	3.50	3.52	0.08	0.16
Lactose	4.55	4.64	4.59	0.09	0.26
Total solids	12.8	12.7	12.7	0.18	0.91
Income over feed cost, US$	9.35	9.52	9.92	0.26	0.06

^{a,b}Means with different superscripts differ ($P < 0.05$).

Key Words: yellow wine lees, lactation performance, nitrogen utilization

318 The effect of a low protein diet, with and without amino acid supplementation, on the performance of lactating dairy Holstein cows.

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Limiting the dietary crude protein (CP) intake in cattle to reduce N excretion is gaining interest, but can negatively affect milk production and the farmers' income (1). This research investigates the supplementation of digestible Methionine (dMET) and Lysine (dLYS) in a low CP diet on milk production of lactating cows in comparison with a control diet and a non-supplemented low CP diet. After an adaptation period of 3 weeks, 36 lactating Holstein cows were randomly divided in 6 homogeneous groups in a balanced crossover design with 3 treatments and 3 consecutive periods of 4 weeks each. The 3 treatments were a control diet (CTRL) with 16.9% CP and a rumen degradable protein balance (RDPB) of around 350g/d; a low protein diet (LOW) with 15.1% CP and a RDPB of around 130g/d; and a low protein diet with similar CP (15,2%) and RDPB level (around 150g/d) but supplemented with 14.7g/d dLYS (55g AjiPro-L) and 4.4g/d dMET (14g Excential Rumenpass MET) (LOW+AA) to reach the same levels as in the CTRL diet. The partially mixed ration of grass, maize and pressed beet pulp was fed ad libitum and supplemented with a fixed amount of concentrates to meet individual needs of 105% of net energy (all treatments) and 105% of digestible protein at the intestines (DPI) for CTRL, whereas only 100% of DPI was met for LOW and LOW+AA. The data were analyzed using a linear mixed model (period*treatment+DIM+(1|cow)). Daily total dry matter intake (DMI) was higher ($P < 0.05$) for CTRL (22.2 ± 0.35kg) than for LOW (21.4 ± 0.35kg), whereas LOW+AA had an intermediate DMI (21.8 ± 0.35kg). Milk yield in the CTRL group (29.3 ± 0.7kg) was higher ($P < 0.05$) than in LOW (28.3 ± 0.7kg), whereas LOW+AA performed intermediate (28.8 ± 0.7kg); similar effects were observed for fat-protein-corrected milk (FPCM). Both LOW diets reduced milk urea excretion and increased N efficiency ($P < 0.05$). Adding extra dLYS and dMET, regarded as the most limiting amino acids (2) to the LOW protein diet showed intermediate milk production results, whereas no supplementation resulted in less ($P < 0.05$) milk in comparison with the CTRL. (1) Schwab & Bröderick, 2017 (2) Bahrami-Yekdangi et al., 2016.

Key Words: amino acids, N excretion, milk production

420 Estimating gastrointestinal tissue use of postruminally absorbed amino acids using a stable isotope-based approach.

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Postruminal amino acid (AA) flow is often believed to be readily available to the animal after it is absorbed across the small intestinal lumen into the bloodstream. However, several studies have demonstrated that AA appearance in the portal drained viscera may vary upwards of 80% relative to what appears in duodenal digesta. As such, gut tissues may utilize absorbed AA before use by other tissues. The objective of this study was to estimate use of Lys, Met and His by gastrointestinal tissues post-absorption. To measure this, 6 dry Holstein cows were used in a 6 × 6 Latin square design in 10-d periods. Cows were all fed a basal ration formulated for high-producing lactating cows. Cows were assigned to 1 of 6 treatments: the basal diet alone (BASE), BASE plus a 54-h abomasal infusion of a mixture of unprotected essential amino acids (EAA), and 4 treatments that consisted of BASE plus inclusions of prototypical ruminally-protected AAs. Ruminally-protected Met (M1, M2, M3, M4), Lys (K1, K2, K3, K4) and His (H1, H2) prototypes were used in 4 treatments to evaluate respective plasma appearances: MKH1 = M1+K1+H1; MKH2 = M2+K2+H2; MK1 = M3+K3; MK2 = M4+K4. On d 10 of each period, an 8-h jugular infusion of a mixture of 13C-labeled AA was administered. Isotope ratios were determined via GC-IRMS for each EAA from collected plasma samples and a dynamic 4-pool model was fit to observed EAA isotope ratios to derive post-absorptive entry rates. Plasma appearance of AAs was derived through linear regression of derived entry rates on dietary or abomasally infused EAA. Plasma appearance of Lys from EAA was 93.7% ($P = 0.06 \pm 0.02$), indicating average first-pass Lys loss was 6.3% if we assume the digestibility of EAA was 100%. First-pass loss of Met and His were 16.2% and 9.30%, respectively, as plasma appearances were 83.8% for Met ($P = 0.22 \pm 0.66$) and 90.7% for His ($P = 0.19 \pm 0.67$). Although gut tissue use may not be the sole contributor to first-pass AA loss, accounting for such post-absorptive factors allows for better prediction of dietary AA utilization in ration balancing models.

Key Words: amino acids, metabolism, modeling

424 Histidine dose-response effects on lactational performance and plasma amino acid concentrations in lactating dairy cows fed a metabolizable protein-deficient diet.

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The objective of this experiment was to determine the effect of increasing dietary His levels on milk production, milk composition and plasma AA concentrations in lactating dairy cows fed a metabolizable protein (MP) deficient diet, according to NRC (2001). Twenty Holstein cows, of which 8 primiparous, [64 ± 12 d in milk, 584 ± 61 kg BW, 43.5 ± 12.4 kg milk yield (MY)], were used in a replicated 4 × 4 Latin square design with four 28-d periods. Treatments were a control diet supplying 1.8% digestible (d)His of MP (His1.8) and the control diet supplemented with rumen-protected (RP) His to provide 2.4, 2.8, or 3.3% dHis of

MP (His2.4, His2.8, and His3.3, respectively). Dry matter intake (DMI) and MY were recorded daily, and milk and blood samples collected at 4 time points on the last week of each experimental period. All data were analyzed using PROC MIXED of SAS with treatment and period in the model. Square and cow within square were random effects. Linear and quadratic effects of His dose were tested. Histidine did not affect DMI (*P* = 0.90; averaging 21.0 kg/d; SEM = 1.95) but increased (*P* = 0.01) MY linearly (37.1, 38.9, 39.2 and 39.1 kg/d, respectively). Histidine had a quadratic effect (P = 0.01) on milk fat concentration (3.62, 3.32, 3.37 and 3.41%, respectively) but did not affect milk fat and energy-corrected milk yields (*P* ≥ 0.33). Lactose concentration and yield as well as milk true protein concentration were not affected (*P* > 0.10) by His. Milk true protein yield was increased linearly (*P* < 0.01) by His (from 1.02 kg/d for dHis1.8 to 1.10 kg/d for dHis3.3; SEM = 0.062). Plasma His concentration increased linearly (*P* < 0.001; 21.0, 27.0, 35.1 and 40.0 μM, respectively) with increasing His dose. In this experiment, RPHis supplementation of an MP deficient diet linearly increased milk yield up to 2.8% dHis supply of MP and linearly increased milk protein yield up to 3.3% dHis supply of MP in lactating dairy cows.

Key Words: rumen-protected histidine, milk production, milk protein

425 Effects of feeding of two rumen-protected methionine supplements on productive performance of lactating Holstein dairy cows. F. Francia[1], M. E. Rodriguez-Prado[1], M. Piera[2], and S. Calsamiglia*[1], [1]*Animal Nutrition and Welfare Service, Univeristat Autonoma de Barcelona, Bellaterra, Spain*, [2]*Centre Veterinari Tona, Tona, Spain*.

Ninety-four multiparous Holstein lactating dairy cows were divided in 3 groups to determine the effect of feeding rumen-protected methionine on milk production and composition. Treatments were a control (CTR) diet, and the same diet supplemented with 11.4 g of metabolizable methionine from either KESSENT M (Kemin Animal Nutrition and Health) or Smartamine M (SMT, Adisseo). After 30 d of treatment, milk production and composition (milk samples taken 3 consecutive days) were determined in wk 6 and 10 postpartum. Cows were fed the same diet formulated to meet NRC recommendations (a corn silage-based diet 46:54 forage to concentrate diet; 17.5% CP, 28.4% NDF, 33.2% starch and 4.6% fat and balanced for Lysine) once a day and milked 3 times/d. Data were analyzed using the PROC GLM procedure of SAS as a completely randomized model. Milk yield (kg/d) was higher (*P* < 0.002) in KESSENT M (46.7) than CTR or SMT (43.9 and 44.5, respectively). The 3.5% fat-corrected milk (kg/d) was numerically higher in KESSENT M and SMT (51.3 and 50.6, respectively) compared with CTR (48.8), but did not reach significance (*P* < 0.11). Milk fat content (%) tended to be higher (*P* < 0.06) in SMT (4.38) than in CTR or KESSENT M (4.16 and 4.14, respectively). Milk protein content (%) was higher (*P* < 0.04) in KESSENT M and SMT (3.09 and 3.11, respectively) compared with CTR (3.04). Similar effects (P < 0.02) were observed for casein (%) (2.40, 2.43 and 2.45 for CTR, KESSENT M and SMT, respectively). Milk fat yield (kg/d) was similar among treatments (1.90), but protein yield (kg/d) was higher (*P* < 0.01) in KESSENT M (1.43) compared with CTR and SMT (1.33 and 1.38, respectively). Casein yield (kg/d) was also higher (P < 0.01) in KESSENT M (1.13) compared with CTR and SMT (1.05 and 1.09, respectively). Both rumen-protected methionine supplements improved dairy cow performance compared with control, but differences between the commercial supplements were also observed.

Key Words: rumen-protected methionine, dairy cow performance

Animal Health: Transition Cow 3

319 Effects of yeast culture supplementation on systemic and polymorphonuclear leukocyte mRNA biomarkers of inflammation and liver function in peripartal dairy cows. N. A. Carpinelli*[1], J. Halfen[1,2], R. Mohan[1], E. Trevisi[3], J. D. Chapman[4], E. D. Sharman[4], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University*, *Brookings, SD*, [2]*Núcleo de Ensino, Pesquisa e Extensão em Pecuaria (NUPEEC), Universidade Federal de Pelotas*, *Pelotas, RS, Brazil*, [3]*Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Science*, *Italy*, [4]*Phibro Animal Health Corporation*, *Teaneck, NJ*.

Feeding yeast culture additives has been attributed to health benefits during the transition period of dairy cows. The objective of this study was to evaluate the effects of feeding a commercial yeast culture (Cellerate Culture Classic HD (YC); Phibro Animal Health, Teaneck, NJ) on blood biomarkers of inflammation and liver function, and gene expression in polymorphonuclear leukocytes (PMNL) in dairy cows during the transition period until 50 DIM. Forty Holstein dairy cows were used in a randomized complete block design from −30 to 50 DIM and blocked according to parity, previous lactation milk yield, and genetic merit. At −30 DIM cows were assigned to a basal diet plus a top-dressed of either 114 g/d of ground corn (CON; n = 20) or 100 g/d of ground corn and 14 g/d YC (n = 20). Cows received the same close-up diet from −30 to calving (0.63 Mcal/kg DM and 12.3% CP) and lactation diet (0.73 Mcal/kg DM and 15.6% CP). Blood samples were collected at −30, −15, 7, 14, and 30 DIM to evaluate biomarkers related to liver function and inflammation. Blood samples for PMNL gene expression were collected at −30, −15, 5, 10, and 30 DIM. The mRNA in PMNL will be analyzed for genes associated with inflammation, cellular receptor, and oxidative stress. Data were analyzed using the MIXED procedure of SAS. Neither prepartal nor postpartal DMI was affected ($P \geq 0.40$) by dietary effects, whereas ECM tended ($P = 0.07$) to be greater in YC than CON cows. Postpartal diseases and conditions were similar ($P = 0.31$) between treatments. There was a trend ($P = 0.07$) for a Diet × Time interaction in GGT (γ-glutamyl transferase), where greater ($P < 0.01$) GGT was observed in YC than CON cows at 30 DIM. Negative acute-phase proteins (APP), albumin ($P = 0.06$) and paraoxonase ($P = 0.12$) tended to be lower in YC than CON cows. The positive APP, haptoglobin[CN1], tended ($P = 0.12$) to be higher in YC than CON. Results do not demonstrate a conclusive dietary effect on liver function or inflammation, while a modest improvement in milk production was observed in YC cows.

Key Words: transition period, inflammation, yeast culture

320 Effect of different fatty acids on the proliferation and cytokine production of peripheral blood mononuclear cells (PBMC) of dairy cows. N. Vanacker*[1,2], R. Blouin[1], C. Ster[2], and P. Lacasse[2,1], [1]*Université de Sherbrooke*, *Sherbrooke, QC, Canada*, [2]*Agriculture and Agrifood Canada*, *Sherbrooke, QC, Canada*.

During the transition period, dairy cows often experience negative energy balance which induces metabolic and immunological disturbances. Our previous work has shown a relationship between the inhibition of immune functions and the increase in blood nonesterified fatty acid (NEFA) concentrations. We evaluated the effect of 11 fatty acids (palmitoleic, myristic, palmitic, stearic, oleic, linoleic, docosahexaenoic, conjugated linoleic, lauric, eicosapentanoic and linolenic acids) as well as a mix that represent the NEFA profile observed during the transition period, at different concentrations (0, 50, 100 and 250 μ*M*) on proliferation and cytokines secretion of lymphocytes. To assess lymphoproliferation, PBMC from 5 healthy cows (166–189 DIM) were isolated, stimulated with the mitogenic lectin concanavalin-A (ConA) and incubated for 72 h with or without fatty acids. As evidenced by flow cytometry, all fatty acids, except lauric acid, significantly reduced proliferation of PBMC ($P < 0.05$) as the doses increased. The most detrimental effect on PBMC was observed with linoleic and stearic acids, which were able to inhibit proliferation at the lowest doses (50 μ*M*). With regard to cytokine secretion, we found that the concentration of interleukin-4 in culture supernatant of ConA-stimulated PBMC was considerably reduced upon exposure to the lowest dose (50 μ*M*) of oleic and palmitoleic acids ($P < 0.05$) for 24 h while a dose of 100 μ*M* was necessary for eicosapentanoic acid, the NEFA mixture and myristic acid ($P < 0.05$). PBMC also showed decreased ($P < 0.05$) secretion of interferon-γ in response to lauric, linolenic, palmitoleic and stearic acids at 50 μM and myristic acid at 100 μ*M*. Overall, our study confirms that NEFA, such as those of 18 carbons, have a negative impact on some lymphocyte functions, whereas others, like lauric acid, have less effects. We did not observed positive effect for any of the fatty acids on the tested immune functions.

Key Words: lymphocytes, cytokine, immunosuppression

321 Selenium-biofortified alfalfa hay supplementation modulates liver and macrophage gene expression in periparturient dairy cows. S. Busato* and M. Bionaz, *Oregon State University*, *Corvallis, OR*.

Heightened oxidative stress is characteristic of the transition from pregnancy to lactation in dairy cows. Supplementation of selenium (Se) can boost the antioxidant response, improving overall oxidative status of transition cows. In a prior study, we demonstrated that feeding transition dairy cows a relatively low amount (1% BW) of Se-biofortified alfalfa hay increased Se concentrations in blood and liver, increased glutathione peroxidase activity in blood and improved the liver function. Se supplementation can also improve the response to mastitis. Thus, we hypothesized that the gene expression profiles in the liver and milk-derived macrophages would be modulated by Se supplementation to reflect these effects. We collected liver biopsies from 10 periparturient Jersey cows (5 supplemented with Se-biofortified hay, 5 fed control hay, for 54 d), at −10 and +10 DIM. Milk samples were collected from the same cows at +10 DIM, and macrophages were isolated using CD14 as a marker. RNA was extracted using RNAeasy (QIAGEN), integrity was measured using a 2100 Bioanalyzer (Agilent), and samples were sequenced using a HiSeq 3500 (Illumina). We detected approx. 10,000 transcripts in both type of samples. Using an FDR of 0.30 as cut-off with JMP genomics (SAS), we detected 247 and 148 DEG (237 and 129 downregulated by Se) in the transcriptome of macrophages and liver, respectively. Bioinformatic analysis was performed using the Dynamic Impact Approach and DAVID. Se conferred changes in metabolism- and replication and repair-related pathways in the liver, including downregulation of sphingolipid metabolism in the postpartum and mismatch repair in pre-partum. In macrophages, Se supplementation inhibited overall metabolic-related pathways, especially lipid and carbohydrate metabolisms (citric acid cycle) and inhibited cell cycle and cell redox homeostasis. Taken together, our results suggest a mild but inhibitory effect of organic Se supplementation on the transcriptome of liver and milk macrophages on transition dairy cows, affecting mostly lipid and carbohydrate metabolism and cell cycle.

Key Words: selenium, peripartum, dairy cow

322 Mycotoxins in forage-based feeds from the United States and Canada. P. N. Gott*[1], E. G. Hendel[1], S. M. Ramirez[1], U. Hofstetter[2], and G. R. Murugesan[1], [1]*BIOMIN America Inc*, *Overland Park, KS*, [2]*BIOMIN Holding GmbH*, *Getzersdorf, Lower Austria, Austria*.

The complex nature of ruminant diets leads to increased risk of broad-spectrum mycotoxin exposure. These harmful secondary fungal metabolites are detrimental to animal health and productivity. This study investigated the occurrence and contamination levels of mycotoxins in several forage-based ruminant feeds. Corn silage, haylage (including grass, alfal-

Table 1 (Abstr. 323). Trends in mycotoxin contamination in US corn grain and corn by-product feeds

	Grain			By-Product Feeds		
Item	2017	2018	2019	2017	2018	2019
N	371	442	224	75	61	44
Mean toxin Count	1.46^{a}	2.13^{b}	2.07^{b}	2.84	2.75	3.04
B-Trich						
Prevalence, %	69	76	80	99	93	100
Concentration,[1] ppb	569^{a} ± 71	1,167^{b} ± 101	879^{b} ± 79	2,026^{a} ± 204	3,175^{b} ± 366	2,763^{b} ± 178
FUM						
Prevalence, %	52	79	76	92	90	100
Concentration, ppb	2,390^{a} ± 256	3,299^{ab} ± 331	3,972^{b} ± 455	1,452^{a} ± 286	1,516^{a} ± 347	3,536^{b} ± 701
ZEN						
Prevalence, %	24	45	47	83	85	95
Concentration, ppb	269^{a} ± 85	310^{b} ± 26	451^{ab} ± 130	189^{a} ± 16	527^{b} ± 65	593^{b} ± 55

[a,b]Values within a row with different letters are different ($P < 0.05$).

[1]Mean ± SEM.

fa, and mixed), and straw samples from across the US and Canada were screened as part of an on-going survey for the presence of 6 major mycotoxin groups: aflatoxins, type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN) and ochratoxin A via LC-MS/MS technique. Samples submitted for analysis due to clinical health or performance concerns were excluded from the data set. Among 81 corn silage samples harvested in 2019, B-Trich were most prevalent (94% positive), ZEN frequently occurred (31%), and FUM were minimally detected (7%). Mean contamination levels (parts per billion, ppb) as-received were 1272 ppb, 216 ppb, and 167 ppb, respectively. Only 5% of silage samples were below the limit of detection (LOD) for all mycotoxins evaluated. Analysis of 14 haylage samples from 2019 crop year (various cuttings represented) revealed B-Trich as the most commonly detected mycotoxin group with 57% of samples positive at a mean concentration of 1607 ppb. Twenty-one 2019 straw samples were submitted for analysis with 100% testing positive for some level of B-Trich and 81% positive for ZEN. Mean B-Trich levels in straw were 2001 ppb while ZEN averaged 640 ppb. Seventy-one percent of straw samples were positive for both B-Trich and ZEN. Preliminary survey results suggest B-Trich and ZEN are the most commonly occurring major mycotoxins in 2019 forage-based feeds including corn silage, haylage, and straw from US and Canada. Increased scrutiny of forage-based feeds for their potential contribution to overall mycotoxin load in the diet is warranted as contamination was both frequent and at high enough levels to pose potential risk to the health, performance, and reproductive success of ruminants.

Key Words: mycotoxin, forage, silage

323 Recent mycotoxin contamination trends in US corn grain and corn by-product feeds. P. N. Gott*[1], E. G. Hendel[1], S. M. Ramirez[1], U. Hofstetter[2], and G. R. Murugesan[1], [1]*BIOMIN America Inc*, *Overland Park, KS*, [2]*BIOMIN Holding GmbH*, *Getzersdorf, Lower Austria, Austria*.

Certain molds produce secondary metabolites called "mycotoxins" which are detrimental to animal health and negatively affect productivity and reproductive performance. As part of an on-going survey of mycotoxin occurrence, samples were screened for the 6 major mycotoxin groups: aflatoxins, type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN) and ochratoxin A via LC-MS/MS technique. The 2019 crop year data were compared with the prior 2 harvest years using Kruskal-Wallis Test (Prism7, GraphPad, La Jolla, CA) and are presented in Table 1. Mean B-Trich contamination (ppb, parts per billion) in corn grain is numerically down in 2019 versus 2018, but remains greater than 2017 levels. In grain, mean ZEN level and prevalence continues to rise while FUM values are consistent with 2018, but elevated vs 2017. Mean toxin count per sample in 2019 corn grain is similar to 2018, while co-contamination in corn by-product feeds remains high, surpassing 3 toxins per sample. In by-products, mean B-Trich level is down numerically vs. 2018 while FUM contamination has more than doubled over the same timeframe. Both ZEN occurrence and mean levels in 2019 by-products are the highest seen in the past 3 years. Preliminary results of the 2019 corn grain and corn by-product feeds survey indicate mycotoxin occurrence and contamination levels are similar to the prior crop year and will continue to challenge animal health and performance as these ingredients are fed out.

Key Words: mycotoxin, corn, by-products

324 Effects of supplemental mycotoxin deactivator on lactation performance of Holstein cows fed natural mixed mycotoxin contaminated feed. N. L. Whitehouse*[1], H. C. Robertson[1], B. K. Kerns[1], S. M. Hollister[1], L. H. P. Silva[1], and S. M. Fredin[2], [1]*University of New Hampshire*, *Durham, NH*, [2]*Adisseo USA Inc*, *Alpharetta, GA*.

Mycotoxin contamination of feed ingredients poses a serious health risk to dairy cows. The objectives of this trial were to determine the effects of a mycotoxin deactivator on lactation performance and plasma leukocytes in dairy cows fed mixed mycotoxin contaminated feed. Twenty-four Holstein cows (147 ± 58 DIM) were used in a replicated randomized block trial for 7 wks. Treatments were 1) negative control diet (NC); 2) positive control diet with mycotoxin exposure from contaminated dried distillers grains (PC); and 3) PC diet plus 30 g/d of mycotoxin deactivator (UNIKE Plus, Adisseo; UP). Milk yield and DMI was determined daily, averaged by wk. Milk samples were collected on the last 3 d of each wk. Blood samples were collected from the coccygeal vein for 3 consecutive d at the end of the covariate wk and wk 3 and 6 for analysis run on the veterinary hematology platform. Data were analyzed using the MIXED procedure of SAS with REPEATED measurements. Significant effects were noted at $P \leq 0.05$. The TMR in the PC diets increased zearalenone from 290 to 683 μg/kg and deoxynivalenol from 2,013 to 4,772 μg/kg compared with NC diet. Intake decreased for cows fed PC compared with NC but improved for cows fed UP, whereas ECM yield was decreased for cows fed PC compared with NC but unaffected for cows fed UP. Cows fed PC and UP had elevated SCS compared with NC. Leukocytes were unaffected by treatment, but monocytes were elevated for cows fed NC and UP compared with PC. Adding a mycotoxin deactivator improved DMI and ECM yield, increase monocytes with no effect on the other immune cells for cows fed natural mixed mycotoxin contaminated feed.

Key Words: leukocytes, mycotoxin

Table 1 (Abstr. 324). Effects of mycotoxin contamination and deactivation on cow performance

				P-value		
Item	NC	PC	UP	Treatment	Week	Treatment × Week
DMI, kg/d	25.3	23.3	24.1	0.09	0.001	0.02
Milk yield, kg/d	41.6	40.7	40.9	0.80	0.001	0.04
ECM, kg/d	40.5	39.2	40.1	0.62	0.001	0.008
SCS	1.3^{b}	1.8^{a}	1.8^{a}	0.01	0.07	0.23
Neutrophils, cells/µL	4,543	4,125	4,739	0.07	0.002	0.82
Lymphocytes, cells/µL	3,316	2,871	3,338	0.69	0.83	0.98
Monocytes, cells/µL	351^{a}	272^{b}	368^{a}	0.01	0.03	0.80
Leukocytes, K/µL	8.5	7.6	8.7	0.26	0.35	0.98

[a,b]Means within rows differ for Trt ($P < 0.05$).

Breeding and Genetics

325 Genomic predictions for milk yield of crossbred dairy cattle. Y. Steyn*[1], D. Gonzalez-Pena[2], N. Vukasinovic[2], D. Lourenco[1], I. Misztal[1], and S. DeNise[2], [1]*University of Georgia*, *Athens, GA*, [2]*Zoetis*, *Kalamazoo, MI.*

The objective of this study was to predict genomic breeding values for milk yield of crossbred dairy cattle under different scenarios using single-step genomic BLUP (ssGBLUP). Genotypes of 89,558 Holstein, 40,769 Jersey and 22,373 crossbred animals were used, of which all Holstein, 9,313 Jersey and 1,667 crossbred animals had phenotypic records. Low density genotypes were imputed to 45k SNP markers. SNP effects were estimated from single-breed evaluations for Jersey (JE), Holstein (HO) and crossbreds (CROSS), and multi-breed evaluations including all Jersey and Holstein (JE_HO) or approximately equal proportions of Jersey, Holstein and crossbred animals (MIX). Direct genomic predictions (DGV) of the validation animals (358 crossbred animals with phenotypes excluded from evaluations) were calculated using the resulting SNP effects. Additionally, breed proportions (BP) of crossbred animals were applied to combine DGV estimated based on each pure breed. The predictivity of DGV was calculated as Pearson correlation between DGV and phenotypes of the validation animals adjusted for fixed effects in the model. Regression of adjusted phenotypes on DGV was used to assess the inflation of DGVs. The predictivity of DGV for CROSS, JE, HO, JE_HO and MIX scenario was 0.50, 0.50, 0.47, 0.50, and 0.46, respectively. Using BP was least successful, with a predictivity of 0.32. The inflation of the DGV for CROSS, JE, HO, JE_HO, MIX and BP scenarios were 1.17, 0.65, 0.55, 0.78, 1.00, and 0.85, respectively. Rather than using BP, the DGVs of crossbred animals should be predicted using ssGBLUP under a scenario that includes pure breed genotypes

Key Words: single-step GBLUP, indirect predictions, SNP effects

326 Genetic and non-genetic factors associated with lactation length in seasonal-calving dairy cow pasture-based systems. M. Williams*[1,2], C. P. Murphy[2], R. D. Sleator[2], M. M. Judge[1], S. C. Ring[3], and D. P. Berry[1], [1]*Department of Animal Bioscience, Animal and Grassland Research and Innovation Centre, Teagasc*, *Moorepark, Fermoy, Co. Cork, Ireland*, [2]*Department of Biological Sciences, Cork Institute of Technology*, *Bishopstown, Co. Cork, Ireland*, [3]*Irish Cattle Breeding Federation*, *Bandon, Co. Cork, Ireland.*

Lactation yield estimates standardized to common lactation lengths of 270-d or 305-d equivalents are commonly used in management decision-support tools and dairy cow genetic evaluations. The use of such measurements to quantify the (genetic) merit of individual cows fails to penalize cows that do not reach the standardized lactation length, or indeed reward cows that lactate for more than 270 or 305 d. The objective of this study was to identify the genetic and non-genetic factors associated with lactation length in seasonal-calving pasture-based dairy cows. A total of 616,350 lactation length records, from 285,598 cows, were used. Linear mixed models were used to investigate the associations between lactation length and calving month, calving day, parity, age at calving, dry period length, calving difficulty score, herd size, heterosis, recombination loss, and breed, as well as to estimate the variance components of lactation length. The median lactation length was 288 d, with 27% of cows achieving lactations > 305 d. Relative to cows calving in January, the lactation of a cow calving in February, March, or April was 4.2, 12.7, and 21.9 d shorter, respectively. The lactation length of a first-parity cow was 7.8, 8.6, and 8.4 d shorter than that of second, third, and fourth parity cows, respectively. Norwegian Red and Montbéliarde cows had, on average, a 4.7 and 1.6-d shorter lactation than Holstein-Friesian cows, respectively. The heritability estimate and coefficient of genetic variation for lactation length were 0.02 and 1.2%, respectively. When ranked on their genetic merit for lactation length, there was a 9.2-d phenotypic difference in lactation length between cows in the top and bottom 20%; demonstrating exploitable genetic variability. Given the vast array of genetic and non-genetic factors associated with lactation length; an approach which combines improved management practices and selective breeding may be the most efficient way to lengthen lactations.

Key Words: lactation length, genetic merit, non-genetic factors

327 β-Casein A2 genetics of organic Holstein dairy cows across the Midwest and Northeast. B. J. Heins*[1], G. M. Pereira[1], L. C. Hardie[2], and C. D. Dechow[2], [1]*University of Minnesota*, *Morris, MN*, [2]*Pennsylvania State University*, *State College, PA.*

The objective of the study was to determine milk, fat, protein production and SCS for β casein A2 genotypes of organic Holstein cows. Holstein cows (n = 382) from 5 dairy herds across the Midwest and Northeast were genomic tested with CLARIFIDE Plus for A2 status. Twenty-eight cows were A1A1 (7%), 165 cows were A1A2 (43%) and 189 cows were A2A2 (49%). In total, 637 lactation records were used with 375 from first parity, 211 from second parity, and 51 from third parity. Daily milk, fat, and protein production and SCS from milk recording were calculated with Best Prediction. Best prediction was used to calculate actual production (milk, fat, and protein) for 305-d lactations. Adjustment was made for age at calving and milking frequency, and records less than 305 d were projected to 305 d. Independent variables for statistical analysis with PROC MIXED included the fixed effects of parity, herd and milk β casein genotype (A1A1, A1A2, A2A2). Cow and birth date were random effects in the statistical model. Milk production was not different ($P > 0.12$) for A1A1 (7,970 kg), A1A2 (7,910 kg), and A2A2 (7,728 kg) cows. Fat plus protein production was not different ($P > 0.14$) for A1A1 (600 kg), A1A2 (591 kg), and A2A2 (581 kg) cows. Furthermore, SCS was not different ($P > 0.44$) for A1A1 (2.68), A1A2 (2.53), and A2A2 (2.61 kg) cows. Results indicate no difference in production and SCS with regard to A1 or A2 genotype in organic dairy herds.

Key Words: genetic selection, organic, A2 milk

328 Genetic traits and β-casein A2 of Holstein and crossbred dairy cattle in an organic and low-input dairy herd. B. J. Heins*[1], G. M. Pereira[1], L. C. Hardie[2], and C. D. Dechow[2], [1]*University of Minnesota*, *Morris, MN*, [2]*Pennsylvania State University*, *State College, PA.*

The University of Minnesota initiated a crossbreeding designed study in 2000. The study continues at the West Central Research and Outreach Center, Morris, Minnesota, and is currently part of the low-input sustainable and organic grazing dairy project. The 2 research herds are composed of 1964 genetic control Holsteins (HO), contemporary HO, ProCROSS composed of Montbéliarde, Viking Red, and HO breeds and GrazeCross cattle composed of Jersey, Normande, and Viking Red breeds. Control HO (n = 107), ProCROSS (n = 385), GrazeCross (n = 233), were compared with contemporary HO (n = 224) for genetic traits and Beta Casein A2 status from genomic testing. Four hundred and seventy animals were genotyped with the GGP Bovine 50K and 295 animals with the GGP Bovine 150K from Neogen Genomics (Lincoln, NE) and 176 animals from the ZL5 Clarifide Plus from Zoetis (Kalamazoo, MI). The Beta Casesin A2A2 genetics were 23% for control HO, 49% for HO, 40% for ProCross, and 53% for GrazeCross. The control HO had a greater percentage ($P < 0.01$) of cows that were A1A1 (27%) than the HO (12%), ProCross (14%) and GrazeCross (8%). Predicted transmitting abilities for genetic traits were from the December 2019 genetic evaluation from the Council on Dairy Cattle Breeding (Bowie, MD). Independent variable for statistical analysis of genetic traits with PROC GLM was the fixed effect of genetic line and A2 status. The control cattle had ($P < 0.01$) lower NetMerit$ (-$758) compared with the contemporary HO (+367), ProCross (-$7), and GrazeCross ($14) cattle, respectively. Within each breed group the A2A2 cattle were similar ($P > 0.52$) to A1A1 cattle for NetMerit$. Future studies will compare the genetic lines with genome-wide association analysis for production and health traits.

Key Words: genetic selection, organic, health

329 ProCROSS calves compared with Holstein calves for calving traits in two research herds. G. M. Pereira*[1,2], L. B. Hansen[1], and B. J. Heins[2,1], [1]*University of Minnesota*, *Saint Paul, MN*, [2]*West-Central Research and Outreach Center*, *Morris, MN*.

ProCROSS crossbred calves (n = 1,393) composed of the Montbéliarde, Viking Red, and Holstein (HO) breeds were compared with HO calves (n = 1,121) for gestation length (GL), calf weight at birth (CW), stillbirth (SB), and calving ease (CE). Calves were born in 2 research herds at the University of Minnesota, a high-input dairy at St. Paul (n = 1,116) and a low-input dairy at Morris (n = 1,398) from January 2009 to December 2019. Twin births were removed. Independent variables for statistical analysis of all traits were the fixed effects of herd, sex of calf, breed group of calf, year-season nested within herd, sire breed of calf nested within the interaction of sex of calf and breed group of calf, and the random effect of cow nested within the interaction of herd and breed group of calf. Primiparous and multiparous calvings were analyzed separately. ProCROSS calves had longer GL ($P < 0.01$) than Holstein calves of 278.4 d and 280.5 d, respectively, from primiparous dams. The CW was higher ($P < 0.01$) for ProCROSS calves (40.4 kg) compared with HO calves (39.4 kg) from primiparous dams. The SB and CE (1 to 5 scale) were not different for ProCROSS and HO calves from primiparous dams. ProCROSS calves had longer ($P < 0.01$) GL (281.3 d) compared with HO calves (279.3 d) from multiparous dams. ProCROSS calves had higher ($P = 0.02$) CW (44.2 kg) compared with HO calves (43.3 kg) from multiparous dams. The SB was not different for ProCROSS and HO calves (10%) from multiparous dams. Furthermore, CE was not different for ProCROSS and HO calves from multiparous dams. Results indicate ProCROSS calves were similar to HO calves for CE and SB; however, GL of ProCROSS calves was 2.1 d longer, and CW was 1.0 kg higher than HO calves. Longer GL or higher CW does not result in higher SB rates or higher CE.

Key Words: crossbreeding, low-input, calving trait

330 ProCROSS and Grazecross calves compared with Holstein calves for calving traits in a low-input dairy herd. G. M. Pereira*[1,2], L. B. Hansen[1], and B. J. Heins[2,1], [1]*University of Minnesota*, *St. Paul, MN*, [2]*West-Central Research and Outreach Center*, *Morris, MN*.

ProCROSS crossbred calves (n = 922) composed of the Montbéliarde, Viking Red, and Holstein (HO) breeds and Grazecross crossbred calves (n = 401) composed of the Normande, Jersey, and Viking Red breeds were compared with HO calves (n = 476) for gestation length (GL), calf weight at birth (CW), stillbirth (SB), and calving ease (CE). Calves were born at the University of Minnesota low-input dairy herd in Morris from January 2009 to December 2019. Twin births were removed. Independent variables for statistical analysis of all traits were the fixed effects of sex of calf, breed group of calf, year-season, sire breed of calf nested within the interaction of sex of calf and breed group of calf, and the random effect of cow nested within breed group of calf. Primiparous (n = 737) and multiparous (n = 1,062) calvings were analyzed separately. ProCROSS calves had longer ($P < 0.01$) GL (280.9 d) than HO calves (277.8 d) but similar GL to Grazecross calves (280.9 d) from primiparous dams. The CW was higher ($P < 0.01$) for ProCROSS calves (39.7 kg) compared with HO calves (38.4 kg) and Grazecross calves (34.4 kg) from primiparous dams. The SB and CE (1 to 5 scale) were not different for ProCROSS, Grazecross, and HO calves from primiparous dams. ProCROSS calves had similar GL to Grazecross calves (282.0 d and 282.1 d, respectively), but crossbred calves had longer GL ($P < 0.01$) compared with HO calves (279.4 d) from multiparous dams. ProCROSS calves had higher ($P \leq 0.01$) CW (42.9 kg) compared with HO calves (41.9 kg) from multiparous dams. Additionally, ProCROSS and HO calves had higher ($P < 0.01$) CW than Grazecross calves (37.2 kg) from multiparous dams. The SB and CE were not different for ProCROSS, Grazecross, and HO calves from multiparous dams. Results indicate crossbred calves were similar to HO calves for CE and SB; however, crossbred calves had mean GL that was 2.9 d longer than HO calves. ProCROSS calves had mean CW that was 1.1 kg higher than HO calves. However, ProCROSS calves had mean CW that was 5.5 kg higher than Grazecross calves, and this was expected because some calves were sired by Jersey bulls.

Key Words: crossbreeding, calving trait, Normande

Dairy Foods: Cheese

331 What is the fate of *Listeria monocytogenes* in various types of Belgian artisanal cheeses? A. Gérard*[1], E. Van Coillie[2], A. Bentaib[3], G. Daube[4], and M. Sindic[1], [1]*Laboratory of Quality and Safety of Agro-Food Products, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium*, [2]*Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Technology and Food Science Unit, Melle, Belgium*, [3]*Quality Partner sa, Herstal, Belgium*, [4]*Faculty of Veterinary Medicine, Food Science Department, FARAH, University of Liège, Liège, Belgium*.

Cheese has generally to be considered as ready-to-eat food allowing the growth of *Listeria monocytogenes*, although cheeses unable to support this growth were already observed. Consequently, the absence of the pathogen in 25 g of cheese has to be guaranteed, before placing it on the market, while up to 100 cfu/g are tolerated for food not allowing the growth of *L. monocytogenes*. The presence of *L. monocytogenes* in cheese can lead to harmful economic consequences for producers. Defining more accurately cheeses allowing or not the growth of *L. monocytogenes* is thus a priority. Predictive microbiology is not an optimal solution, since artisanal cheeses present specific characteristics that are not taken into account by current models. Challenge-tests seem more accurate. The goal of this study was to assess the growth potential of *L. monocytogenes* in cheeses from 32 artisanal factories using challenge-tests. Were considered: (a) unripened cheeses (12), (b) mold-ripened soft cheeses (4), (c) smear-ripened soft cheeses (4) and (d) ripened semi-hard cheeses (12). The number of batches to test was determined using Sym'Previus, an online tool for growth predictions in food microbiology. A cocktail of 3 strains was inoculated in cheeses, targeting a contamination of 100 cfu/g. Cheeses were stored at refrigeration temperature during the whole shelf-life. Growth potentials were calculated as the difference between median contaminations at the use-by date and at the first day of storage, as recommended by the European Union Reference Laboratory for *L. monocytogenes*. Twenty-three cheeses out of 32 did not allow the growth of *L. monocytogenes*, i.e., the growth potential was $\leq 0.5 \log_{10}$ cfu/g. It was the case of all unripened cheeses ($-1.0 \pm 0.3 \log_{10}$ cfu/g on average), meaning that Belgian unripened cheeses should not represent a threat for food safety. On the opposite, soft cheeses allowed growth of *L. monocytogenes* up to 4.5 $\log_{10}$ cfu/g. Regarding semi-hard cheeses, a huge inter- and intra-batch variability was observed. In the latter case, recommended method for growth potential calculation underestimated the growth and led to inaccurate conclusions concerning product safety.

Key Words: *Listeria monocytogenes*, cheese, challenge test

332 Efficacy of bioengineered nisin derivatives H27/31K in combination with phage endolysin PlyP100 to eliminate *Listeria monocytogenes* in queso fresco. L. A. Ibarra-Sánchez*, W. Kong, T. Lu, and M. J. Miller, *University of Illinois at Urbana-Champaign, Urbana, IL.*

Listeria monocytogenes is a food-borne pathogen of concern in fresh Hispanic-style cheeses, such as queso fresco (QF). Nisin's ability to inhibit *L. monocytogenes* is well-known, but its activity is limited in QF. The objective of this study was to evaluate the antilisterial efficacy of bioengineered nisin H27/31K, alone and in combination with endolysin PlyP100 in QF. Nisin H27/31K (histidine at position 27 and 31 replaced with lysine) was produced in *Lactococcus lactis* MG1363, an engineered strain having the nisin biosynthetic pathway. Commercial nisin (nisin A) and H27/31K were evaluated to determine their minimum inhibitory concentration (MIC) and residual nisin after 24 h exposure to pH 7 + 22% milk fat at 37°C. His-tagged PlyP100 was overexpressed in *Escherichia coli* and subsequently purified. Nisin A, H27/31K and PlyP100 were added to QF at the following concentrations: 250 μg/g H27/31K with or without 2.5 U/g PlyP100, and 250 μg/g nisin A. Cheese curds were inoculated with ~3.5 log cfu/g of *L. monocytogenes* cocktail of 5 different foodborne outbreak-associated strains. *L. monocytogenes* cells were enumerated by spread plating on PALCAM (polymyxin-acriflavine-LiCl-ceftazidime-esculin-mannitol) agar supplemented with ceftazidime, across 28 d of storage at 4°C. All experiments were repeated 2 times with duplicated samples. H27/31K showed reduced antilisterial activity (MIC range: 12.5 - 50 μg/mL) compared with nisin A (MIC range: 1.56 – 6.25 μg/mL). After 24 h of exposure to pH 7 + 22% milk fat, > 96% residual nisin H27/31K was observed, but residual nisin A was not detected. H27/31K reduced initial viable counts of *L. monocytogenes* in QF by up to ~1.5 log cfu/g. PlyP100 exhibited a strong listeriostatic effect in QF over 28 d of cold storage. The treatment combining H27/31K and PlyP100 in QF achieved *L. monocytogenes* reduction below the detection limit of plating, and, the pathogen was not recovered after enrichment in all QF samples treated with that antimicrobial mixture. In conclusion, our results demonstrate that nisin H27/31K combined with PlyP100 can be used to eradicate *Listeria* in QF.

Key Words: queso fresco, *Listeria monocytogenes*, nisin bioengineering

333 Physical and sensorial characteristics of raw milk cheeses and pasteurized milk cheeses from sheep supplemented with sunflower seed silage. E. Cardoso-Gutiérrez[1], A. C. Narvaes-López[1], L. E. Robles-Jiménez[1], M. d. l. Á. Colín-Cruz[1], M. González-Ronquillo[1], and E. Vargas-Bello-Pérez*[2], [1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, México*, [2]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark*.

The objective of this study was to evaluate the physical and sensorial characteristics of raw milk cheeses (RM) and pasteurized milk (PM) cheeses from sheep supplemented with sunflower seed silage (SFS). Six East Friesian ewes were supplemented with SFS (5% of dry matter) for 8 weeks. Three cheeses (1 kg/each) per treatment (RM and PM) were manufactured every 2 weeks (12/treatment), and allowed to mature for 180 d. After maturation times, sensory analyses were performed with a panel composed of 50 untrained judges. Evaluations on odor, color, texture, general taste and overall acceptability used a 5-point hedonic scale (1 = lowest and 5 = highest; intensities). Likewise, pH, colorimetric, shear force and quantification of mesophylls, coliforms, fungi and yeasts were determined. Data were analyzed in a completely randomized design using GLM procedure from SAS. Scores for odor (3.10 ± 0.20), color (3.57 ± 0.38), texture (3.32 ± 0.58), flavor (3.12 ± 0.24), and acceptability (3.12 ± 0.21) were similar. The pH was more acidic ($P < 0.01$) for RM cheeses (4.78) compared with PM (5.51). Treatments were similar in shear force (2.19 ± 0.16 kg). Lightness (L*; 73.9 ± 2.21), redness (a*; −1.36 ± 0.78), chroma (C*; 14.13 ± 9.13) and hue (H*; 94.26 ± 2.78) were similar between treatments. Compared with PM, the yellowness was higher ($P < 0.05$) in RM cheeses (b*; 16.82 vs. 24.89). Counts for mesophylls, coliforms, and yeasts were similar between treatments. Overall, RM cheeses from sheep supplemented with SFS have similar sensory characteristics than PM cheeses but lower pH and more intense yellowness.

Key Words: raw milk, sheep, sunflower silage

334 Manufacture of culture-based acid curd using micellar casein concentrate. A. R. A. Hammam* and L. E. Metzger, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Micellar casein concentrate (MCC) is a high protein ingredient produced by microfiltration of skim milk. It has an elevated level of casein as a percentage of total protein as compared with skim milk. Acid curd is a protein concentrate, which is produced by precipitating the casein at the isoelectric point (pH = 4.6) using starter cultures or acids without rennet. Acid curd is typically manufactured from skim milk in a process similar to Cottage cheese manufacture. However, this results in the production of acid whey which is difficult to utilize. We theorize that acid curd could be produced from MCC instead of skim milk which could improve the efficiency of manufacture and allow for removal of the whey protein before acid curd manufacture. The objective of this study was to utilize MCC to manufacture of acid curd using starter cultures. The MCC (pH~6.6) was prepared and standardized by mixing milk permeate, water, and MCC powder to produce a solution with 13.0% solids, 9.0% protein, and 2.0% lactose. Thermophilic cultures were added (0.005%) to the MCC and incubated at 43°C. The fermentation time was approximately 15 h to decrease the pH to 4.6. After reaching pH 4.6, the curd was cut and mixed gently during heating to 50°C in 1 h. The whey was subsequently drained and the curd was washed with water and then pressed. After pressing, the curd was analyzed for solids, protein, lactose, lactic acid, ash, and mineral profile. The moisture adjusted yield efficiency was also calculated. This trial was repeated 4 times. The mean was 24.9%, 23.0%, 0.90%, and 97.4% for solids, protein, ash, and moisture adjusted yield efficiency, respectively. The whey had 0.58% lactose and 1.43% lactic acid. The calcium and phosphate content of the acid curd was 0.19% and 0.12%, respectively. No significant differences ($P > 0.05$) were detected in the composition of the acid curd made from the 4 replicates of the MCC. We conclude that MCC can be utilized in manufacture of acid curd with starter cultures. The culture-based acid curd will be utilized as an ingredient in imitation Mozzarella type cheese in subsequent studies.

Key Words: micellar casein concentrate, acid curd, imitation mozzarella cheese

335 Manufacture of designer milk powder for recombined cheeses.

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Our ultimate goal is to minimize whey drainage in cheesemaking by boosting total protein content in rehydrated milk. Commercial milk protein powders pose 2 major challenges in preparing high protein recombined milk – poor solubility at high protein and slower cheese ripening in semi-hard cheeses due to significant amount of whey proteins. Thus, we made a whey-protein depleted designer powder (WDSCC) with higher lactose and lower calcium (Ca) to aid powder hydration and solubility and compared with 7 commercial powders (MPC 80, MPC 85, MPI 85, MPI 85 Low Lactose, MPI 90 and 2 micellar casein powders) in terms of composition, solubility and rennet coagulation properties. All experiments were replicated (n = 3) and multiple comparison ($\alpha = 0.05$, Duncan test) were used for statistical analyses. The WDSCC powder was made by microfiltration/diafiltration (MF/DF) of pasteurized skim milk at 24°C. The MF retentate (casein, CN) was acidified and ultrafiltered/diafiltered (UF/DF) at pH 5.5 to get CN with reduced colloidal Ca phosphate. The UF permeate was nanofiltered (NF) to retain whey proteins and permeate lactose and monovalent minerals. Edible-grade lactose, NF permeate and, calcium-depleted CN were blended together, and spray dried to obtain the designer powder - soluble casein concentrate. The WDSCC powder manufacture was replicated twice. WDSCCs have CN: true protein ratio of 0.95 as compared with 0.86–0.92 in commercial powders. WDSCC contained higher lactose (24% versus 2–6%, $P < 0.05$) and lower total Ca/g protein (15mg versus 21–25mg, $P < 0.05$) than commercial high-CN powders. Powders were rehydrated to 7.5, 10 and 12.5% total protein using a magnetic stirrer at 800 rpm for 1 h. Solubility and rennet coagulation (using small amplitude oscillatory rheology) were measured at each protein content. Rehydration at 20°C for 10% protein, resulted in commercial powders having lower solubility (average ~60%) than WDSCCs (83–91%) ($P < 0.05$). As protein concentration was increased, all commercial powders immediately gelled during rehydration but, WDSCCs were easily rehydrated to 12.5% and 15% protein. As WDSCCs were Ca-depleted, no rennet gels formed at ≤ 12.5% protein without adding Ca. 15% WDSCC coagulated regardless of Ca addition. At higher protein, WDSCCs have superior solubility, hydration and rennet coagulation than commercial powders but it has higher lactose content. In future studies, we will evaluate the potential of WDSCC powder in preparing high protein recombined milk for wheyless cheesemaking.

336 Impact of lactose standardization and curd types on the properties of direct-salted Gouda cheese.

Y. Gong*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], M. E. Johnson[2], and J. A. Lucey[1,2], [1]*University of Wisconsin–Madison*, *Madison, WI*, [2]*Wisconsin Center for Dairy Research*, *Madison, WI.*

Direct-salted Gouda cheese allows cheese manufacturers to produce Gouda cheese using existing Cheddar equipment. Our previous survey on commercial US Gouda cheeses found that block Gouda was more acidic and lacked desirable melt attributes compared with the traditional Gouda. To improve the sensory and functional properties of direct-salted Gouda cheese, we studied the impact of lactose standardization (LS) and curd types (stirred curd (SC) or milled curd (MC)) on cheese properties. Milk with 2 different lactose contents (~4.4% (control) and ~2.2% (LS)) were prepared using ultrafiltration and water was added to the LS milk. Four types of cheese (controlSC, controlMC, LSSC and LSMC) were made (n = 5) using milk containing similar casein content (~3.1%) and casein-to-fat ratio (0.7). They were ripened at 10°C for 10 d and then 4°C for 3 mo. Composition, textural and sensory analyses were performed after 1-d, 2-wk, 1-mo and 3-mo of ripening. Cheese functionality was assessed using texture profile analysis (TPA) and dynamic low-amplitude oscillatory rheology. Sensory Spectrum and quantitative descriptive analysis were conducted with 10 trained panelists to evaluate flavor, texture, shred attributes, and pizza performance. Multiple comparison ($\alpha = 0.05$, Duncan test) and split-plot design were used for statistical analyses. The 4 cheeses had similar composition except that LS cheeses had slightly lower moisture by 1%. LS Gouda had higher pH values, lower lactic acid contents and lower TPA hardness values than control Gouda during ripening. Rheological parameters, maximum loss tangent (MLT) values were impacted by both LS and curd type. Sensory acid scores were lower in unmelted LS cheeses than unmelted control cheeses at 2 wk. When baked on pizza at 1 mo, all 4 cheeses melted completely and had similar blister quantity, strand thickness, and strand length. The use of LS effectively controlled the pH values and reduced the acid flavor in block Gouda cheese. LS had a greater effect on cheese flavor and functionality than curd type.

Key Words: block Gouda cheese, lactose standardization, curd type

337 Evaluation of calf body weight with a partial-weigh scale on an automatic calf feeder. B. J. Heins* and K. T. Sharpe, *University of Minnesota*, *Morris, MN*.

The objective of this study was to evaluate a forefront weigh scale on stalls attached to an automatic calf milk feeder system to determine the accuracy of the scales on calf BW. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN, dairy. Forty-seven Holstein and crossbred calves were fed either 8 L/d or ad lib milk from September 2019 to February 2020. Calves were introduced to the Holm & Laue HL100 Programmable Calf Feeder (Holm & Laue GmbH & Co KG, Westerronfeld, Germany). Calves were weaned from the automated feeder at 56 d. Weight scales were attached to 4 hygiene feeding stalls on the automated calf feeder. The calf weights from the automated calf feeder were compared with the gold standard calibrated electronic scale (Avery Weigh-Tronix LLC, Fairmont, MN scale head). Calves were weighed once per week. The automated calf feeder had a radio frequency identification reader place on the left side of the hygiene stall and scale for calf identification. The weigh scale was a forefront scale that calves had to stand on the scale with both front hooves to access milk. Data were downloaded from the CalfGuide management software. The associations of the daily weights from the automated feeder scale and the electronic scale were determined with Pearson correlation (PROC CORR of SAS) and Bland-Altman plots. Furthermore, PROC GLM of SAS was used to regress electronic BW on the automated calf feeder weight for each calf. A total of 587 weight observations were used for statistical analysis. The Pearson correlation of electronic weight compared with the automated feeder weight was 0.989 ($P < 0.0001$). The concordance correlation coefficient was 0.988. Correlations for individual calves ranged from 0.971 ($P < 0.001$) to 0.999 ($P < 0.001$). The slope of the regression line was 0.93 ($T < 0.01$) and 95% confidence interval was 0.916 to 0.947. A mean bias of 0.279 kg was observed from the Bland-Altman plots. The results suggest that there is potential for the forefront weigh scale to be used on automated calf feeders to support management decision making.

Key Words: automated feeder, calves, body weight

338 Effects of milk replacer feeding rate and frequency on metabolic responses of dairy calves during summer and winter. R. M. Orellana Rivas*, T. Rodrigues, J. Silveira, V. Lacerda, J. Gao, D. Ferreira de Araújo, J. Souza, T. N. Marins, J. K. Bernard, and S. Tao, *University of Georgia*, *Tifton, GA*.

Holstein calves (n = 48/season) were enrolled at 7 d of age in the summer (June to Aug, BW = 40.6 ± 0.7 kg) and winter (Nov to Jan, BW = 41.9 ± 0.8 kg) to evaluate the effect of milk replacer (MR) feeding rate (FR) and frequency (FF) on metabolic responses. In each season, calves were randomly assigned to 1 of 4 treatments (TRT) in a 2 × 2 factorial arrangement including 2 FR (0.68 [LOW] or 0.79 kg of solid/d [HIGH] of a 26% CP and 17% fat MR) and 2 FF (2 × [0700 and 1600 h] or 3 × [0700, 1600, and 2200 h]). Calves were managed similarly and housed in polyethylene hutches with sand bedding. Milk replacer (12.5%) was fed until d 42 when FR was reduced by half and offered 1 × /d (0700 h) for 7 d. Average ambient temperatures were 23.3 ± 2.4 in summer and 10.5 ± 5.5°C in winter. A subset of calves (n = 8/TRT/season) was subjected to an intravenous glucose tolerance test (GTT) on d 28 and insulin challenge (IC) on d 29 of age at 1000 h. Plasma was collected at −15, −5, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120 min relative to glucose (GLC) or insulin (INS) infusion to analyze GLC, nonesterified fatty acids (NEFA) and INS. Data were analyzed using PROC GLM of SAS. Following GTT in both seasons, 3 × reduced ($P \leq 0.05$) INS increment and area under the curve (AUC) than 2 × . Calves fed HIGH tended to have lower ($P = 0.07$) GLC AUC from 0 to 30 min following GTT than LOW only during winter. In both seasons, INS clearance following IC was similar among TRT ($P > 0.10$). During IC in the summer, 3 × lowered ($P < 0.01$) GLC AUC and NEFA AUC from 0 to 30 min than 2 ×; and LOW with 2 × lowered NEFA AUC from 0 to 60 or 0–120 min than LOW with 3 ×, both of which were similar to HIGH regardless of FF (FF × FR: $P = 0.04$). During IC in the winter, 3 × lowered ($P \leq 0.03$) GLC AUC from 0 to 60 or 0–120 min compared with 2 ×, and HIGH calf with 3 × had lowest decrement of NEFA and greatest AUC from 0 to 30 or 0–60 min than other TRT (FF × FR: $P \leq 0.04$). In conclusion, increasing FF from 2 to 3 × /d reduced pancreatic INS secretion but improved INS dependent GLC uptake in both summer and winter, but the inhibitory effect on lipolysis was inconsistent between seasons.

Key Words: calf, season, metabolism

339 Effects of milk replacer feeding rate and frequency on basal metabolism and abomasal emptying of dairy calves during summer and winter. R. M. Orellana Rivas*, T. Rodrigues, J. Silveira, V. Lacerda, J. Gao, D. Ferreira de Araújo, J. Souza, T. N. Marins, J. K. Bernard, and S. Tao, *University of Georgia*, *Tifton, GA*.

To examine the effect of milk replacer (MR) feeding rate (FR) and frequency (FF) on abomasal emptying and metabolism, Holstein calves (n = 48/season) were enrolled at 7 d of age during summer (June to Aug, BW = 40.64 ± 0.65) and winter (Nov to Jan, BW = 41.86 ± 0.75). Within season, calves were randomly assigned to 1 of 4 treatments in a 2 × 2 factorial arrangement including 2 FR (0.68 [LOW] or 0.79 kg of solid/d [HIGH] of a 26% CP and 17% fat MR), and 2 FF (2 × [0700 and 1600 h] or 3 × [0700, 1600 and 2200 h] daily). Calves were managed similarly and housed in polyethylene hutches using sand bedding. Milk replacer (12.5%) was fed until d 42 when FR was reduced by 50% and fed 1 × /d (0700 h) for 7 d. The ambient temperature averaged 23.3 ± 2.4°C for summer and 10.5 ± 5.5°C for winter. Plasma was collected weekly at 1400 h to analyze metabolites and insulin. Acetaminophen (ACE, 50 mg/kg of BW) mixed with MR were fed to a subset of calves (0700 h, n = 10/treatment/season) on d 21. Plasma was collected at −15, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 420, and 480 min relative to feeding to analyze ACE. The time for plasma ACE to reach maximum (Tmax) was used to evaluate the abomasum emptying rate. Data were analyzed using the mixed procedure of SAS. During summer, feeding HIGH increased plasma glucose and decreased β-hydroxybutyrate (BHB) compared with LOW ($P \leq 0.03$), and feeding 3 × decreased plasma insulin but increased BHB compared with 2 × ($P \leq 0.02$). In the winter, feeding HIGH increased plasma glucose concentration compared with LOW ($P = 0.04$) and feeding 3 × reduced plasma insulin and nonesterified fatty acids concentrations ($P \leq 0.01$) relative to 2 × . Tmax of calves fed 2 × was longer ($P < 0.01$) than 3 × during summer (233 vs.172 min) and winter (252 vs. 188 min). In conclusion, increasing FF accelerated abomasal emptying and reduced insulin release in both seasons, but FF affected basal metabolites inconsistently between seasons. Feeding higher amounts of MR consistently increased plasma glucose but only decreased BHB during summer.

Key Words: calf, metabolites, season

340 Can the threshold on absolute fat residual improve the reliability of milk mid-infrared-predicted traits without using reference values? L. Zhang*[1], C. F. Li[2], F. Dehareng[3], C. Grelet[3], F. Colinet[1], N. Gengler[1], Y. Brostaux[1], and H. Soyeurt[1], [1]*TERRA Teaching and Research Centre, University of Liège-Gembloux Agro-Bio Tech*, *Gembloux, Belgium*, [2]*Hebei Livestock Breeding Station*, *Shijiazhuang, China*, [3]*Valorisation of Agricultural Products Department, Walloon Agricultural Research Centre*, *Gembloux, Belgium*.

Many traits are currently predicted using milk mid-infrared (MIR) spectrometry. However, those predictions can be erroneous for many reasons such as wrong milk collection, bad storage, inappropriate spectrometers management or calibration equation. Comparing predictions and reference values allows detecting those problems. However, preparing and analyzing reference samples are expensive, time consuming and sometimes difficult, especially for indirect traits like CH_4. Therefore, it is relevant to develop approaches based on predictions to detect abnormal values. This work attempted to study the interest of using a threshold of absolute fat residual to detect abnormal MIR predictions. A total of 346,818 milk MIR records were collected from Chinese Holstein cows and analyzed by Bentley FTS spectrometers. The fat content predicted by the manufacturer model being corrected for the bias and slope were assumed to be the control value. From standardized spectra, a second fat content was externally predicted. The working hypothesis is that this content as only based on spectral data will reflect problem/noise present in the spectral data. The absolute residual fat was calculated as the absolute difference between internal and external predictions of milk fat content. The improvement of reliability was assessed using the difference of root mean square error (RMSE) before and after applying a threshold of 0.3 g/dL of milk for the fat residual. RMSE differences for protein, monounsaturated (MFA), saturated (SFA), and unsaturated (UFA) fatty acids were 0.003, 0.023, 0.014, and 0.024 g/dL of milk, respectively. The correlation coefficient between the internal and external predicted phenotypes nearly stayed constant: 0.96, 0.95, 0.97 and 0.95 for protein, MFA, SFA, and UFA, respectively. The use of a threshold based on milk fat residual allowed detecting abnormal predictions but as those values were not so frequent, RMSE and correlation values were not deeply impacted. This cleaning is therefore of interest for dairy herd improvement organizations to ensure the quality of their MIR spectral database.

Key Words: milk components, MIR, prediction reliability

341 Assessment of within- and between-day variability of individual cow milk urea nitrogen. K. F. Reed* and E. M. Wood, *Cornell University*, *Ithaca, NY.*

Milk urea nitrogen (MUN) is a non-invasive metric that has potential to inform cow nutrition and reproduction management decisions because of its demonstrated relationship to plasma urea nitrogen and urinary urea nitrogen. However, before recommendations for individual cow management decisions based on MUN can be developed, we need to understand the extent to which this metric varies within and between days. Previous work suggests PUN/MUN measurements taken within the same day could differ by ≥2 mg/dL but the authors are not aware of any estimates of between day variation in individual cow MUN. Thus, our objective is to estimate the within day and between day variability of MUN throughout lactation. Milk samples were collected 3 x per day for 7 consecutive days from 16 multiparous cows at 2 periods in lactation (average DIM 40, 140). Samples were preserved and refrigerated before they were sent to a commercial lab for midinfrared spectroscopy analysis of MUN within 48 h. We fit linear mixed models of induvial cow MUN observations averaged daily (Mod1) and from each milking (Mod2) including random effects for day and cow within period. A fixed-effect parameter for the change in CP content of the diet between periods was included in both models. In addition, the fixed-effect of milking time was include in Mod1. Parameter estimates in the table indicate average MUN in P1 was similar for both models (~8.2 mg/dL, β_0) with about 1 mg/dl change for each percentage point change in diet CP (% DM, β_{CP}). Estimates for the random effects of cow and day were also similar between models and suggest that MUN samples taken from the same cow on different days is ~1 mg/dL with 40% (Mod2) and 50% (Mod1) of the variation attributable to the cow. The fixed effect of milking time suggests the 2nd milking (M2) is expected to be 0.44 mg/dL higher than the 1st (M1) and 3rd (M3) milking (β_{M2}).

Key Words: MUN, variation

Table 1 (Abstr. 341). Parameter estimates (SD) of linear mixed model results

	β_0	β_{CP}	β_{M2}	β_{M3}	σ_{Cow}	σ_{Day}	σ_{Res}
Mod1	8.35 (0.298)	1.05 (0.387)			0.880	0.500	0.742
Mod2	8.20 (0.306)	1.07 (0.391)	0.439 (0.0947)	0.0403 (0.0963)	0.909	0.503	0.975

Ruminant Nutrition: General

342 Effects of *Lactobacillus plantarum* as direct-fed microbials on the ruminal microbial community composition using a dual-flow continuous culture system. H. F. Monteiro*[1], P. Fan[1], X. Dai[2], J. Arce-Cordero[1], B. C. Agustinho[1,3], R. R. Lobo[1], A. L. J. Lelis[4], V. L. N. Brandão[1], A. Faccenda[3], A. S. Avila[5], R. Restelatto[6], L. G. Silva[7], K. C. Jeong[1], and A. P. Faciola[1], [1]*Department of Animal Sciences, University of Florida*, *Gainesville, FL*, [2]*U.S. Dairy Forage Research Center, ARS-USDA*, *Madison, WI*, [3]*Departamento de Zootecnia, Universidade Estadual de Maringá*, *Maringa, PR, Brazil*, [4]*Departamento de Zootecnia, Universidade de São Paulo*, *São Paulo, SP, Brazil*, [5]*Departamento de Zootecnia, Universidade Estadual do Oeste do Paraná*, *Marechal Cândido Rondon, PR, Brazil*, [6]*Departamento de Zootecnia, Universidade Federal do Paraná*, *Curitiba, PR, Brazil*, [7]*Departamento de Zootecnia, Universidade Estadual Paulista*, *Jaboticabal, SP, Brazil*.

The objective of this study was to evaluate the ruminal microbial community composition changes by feeding *Lactobacillus plantarum* as direct-fed microbials (DFM) in high-producing dairy cows' diets. A dual-flow continuous culture system was used in a replicated 4x4 Latin square design. A basal diet was formulated containing 16% protein and 28% starch and the treatments were: the basal diet without DFM (CTRL); a mix of *Lactobacillus acidophilus* at 1×10^9cfu/g and *Propionibacterium freudenreichii* at 2×10^9 cfu/g (MLP = 0.01% of diet DM); and 2 levels of *L. plantarum* at 1.35×10^9 cfu/g (L1 = 0.05% and L2 = 0.10% of diet DM). Bacterial samples were collected at 0, 2, 4, 6, and 8 h after feeding from the effluent containers; a composite of all time points was made for each fermenter. The microbial community composition was analyzed through sequencing the V4 region of the 16S rRNA gene using Illumina MiSeq platform. Sequencing data were analyzed on QIIME and statistical analysis was performed with R and SAS; orthogonal contrasts were used to compare treatments. The most abundant phyla from all sequences were Bacteroidetes and Firmicutes, with 42% and 34% of relative abundance, respectively. At the family level, all DFM treatments decreased the relative abundance of *Lachnospiraceae* ($P = 0.03$), *Pirellulaceae* ($P = 0.05$), and *Paracaedibacteraceae* ($P = 0.02$) populations, while *L. plantarum* inclusion positively affected the abundance of *Anaeroplasmataceae* ($P = 0.04$). Interestingly, the *Lactobacillaceae* family was not detected in our analysis, which may indicate a low survival rate of the tested DFM in the ruminal fluid. Relative abundance of 9 genera (mostly fibrolytic and proteolytic) decreased with the DFM inclusion, with emphasis on *Butyrivibrio spp.* ($P = 0.01$), while 1 genus increased with DFM inclusion (*Ruminococcaceae UCG-002;* $P = 0.02$). These findings help to explain why DFM inclusion decreased NH_3-N concentration, while pH and lactate concentration were not affected by treatments.

Key Words: 16S rRNA, *Butyrivibrio*, *Lactobacillus acidophilus*

343 In situ rumen degradability and intestinal digestibility of rumen-protected choline sources. C. Faturi[2], F. Francia[1], M. E. Rodriguez-Prado*[1], and S. Calsamiglia[1], [1]*Animal Nutrition and Welfare Service, Univeristat Autonoma de Barcelona*, *Bellaterra, Spain*, [2]*Universidade Federal Rural de la Amazonia*, *Belem, Brazil*.

The objective of this study was to determine and compare rumen degradability and in vitro intestinal digestibility of 3 ruminally protected choline (RP-Chol) products. Rumen-protected choline products were CholiGEM (Kemin Animal Nutrition and Health), Product R and Product N and contained 5.75, 3.23 and 2.71% of nitrogen (% DM). Rumen microbial degradability was determined using the in situ nylon bag technique. Samples (2.02 ± 0.018 g) of each RP-Chol product was weighed into nylon bags and incubated in the rumen of a Holstein dry cow for 0, 2, 4, 8, 16, 24 and 48 h, in duplicate bags and in 2 consecutive periods. Ruminal degradability was calculated with a simple exponential function ($Y = a+b*(1-\exp(-ct))$), where a was the amount of N disappearing from the bag at 0 h; b was the potentially degradable fraction; and c was the degradation rate. The effective degradability of N (EDN) was calculated as: EDN,% = $a+[(b*c)/(c+k)]$; where k (10%/h) is the estimated rate of outflow from the rumen, and a, b, and c are the same as described earlier. For the determination of intestinal digestibility, the in vitro 3-step procedure was used. The undegraded residue after 12 h incubation in the rumen was incubated in vitro in HCl pepsin at pH 2.0, 38°C for 1 h, followed by a phosphate-pancreatin buffer at pH 7.0, 38°C for 24 h. Fraction a was 27.6, 0.4 and 24.7%; fraction b was 57.1, 5.8 and 73.6%, and fraction c was 0.032, 0.002 and 0.081 /h for CholiGEM, Product R and Product N, respectively. Effective ruminal microbial degradation was very low in Product R (0.5%), moderate in CholiGEM (41.4%) and relatively high in Product N (57.6%). However, intestinal digestion was low in Product R (12.2%) compared with CholiGEM (98.4%) and Product N (80.9%) resulting in the highest bioavailability for CholiGEM (57.6%), intermediate for Product N (34.3%) and lowest for Product R (12.1%). Determination of the bioavailability of RP products requires the evaluation of rumen degradability and intestinal digestibility.

Key Words: rumen-protected choline, ruminal degradability, intestinal digestibility

344 The effect of sanguinarine supplementation on villus height and crypt depth in calves. N. Stepanchenko*[1], H. K. J. P. Wickramasinghe[1], E. A. Horst[1], J. V. V. Silva[1], M. R. O'Neil[1], S. Bas[2], F. R. B. Ribeiro[2], and J. A. D. R. N. Appuhamy[1], [1]*Department of Animal Science, Iowa State University*, *Ames, IA*, [2]*Phytobiotics North America LLC*, *Cary, NC*.

Previous studies have shown that sanguinarine supplementations improved intestinal morphology and growth performances of piglets. The objective of this study was to investigate whether the supplementation of a *Macleaya cordata* extract (MCE) with 1.5% of sanguinarine would affect the villus height and crypt depth in calves. Twenty (10 male and 10 female) calves of 6 ± 2 d of age were matched for age, BW, sex, and assigned to 2 groups: control (CTL) and MCE-supplemented (10g and 20g/calf/d pre- and post-weaning respectively). Calves were housed individually in hutches and bottle-fed with milk replacer (12% solid in 6.0 L/d) until 49 d of age. The MCE dose was delivered in milk replacer during the pre-weaning period and was top-dressed on starter feed post-weaning. MCE was fed for a total of 90 d (96 ± 2 d of age). On d 91, calves were euthanized and samples of duodenum, jejunum (15 and 80 cm distal to the pyloric sphincter, respectively), and ileum (15 cm proximal to the ileocecal junction) were obtained. A digital camera attached to an inverted microscope was used to obtain images from 3 non-overlapping fields per sample at 10 × magnification. Villus height and crypt depth were measured using ImageJ software. Average measurements across the 3 fields were used in the statistical analysis using a model including fixed effects of treatment, sex, the intestinal region, and interaction between treatment and the region, and random effect of calf. Villus height changed with treatment ($P = 0.007$) and intestinal region ($P = 0.030$). Villus height in the ileum was greater than that in the jejunum ($P = 0.043$) and duodenum ($P = 0.068$). Compared with CTL, MCE had taller ($P < 0.030$) villi in the duodenum (570 vs 450 μm) and ileum (623 vs 522 μm). Crypt depth did not vary among the regions ($P = 0.301$). The CTL were related to greater crypt depth in the ileum (550 vs 464 μm) than SG. The villus height: crypt depth ratio in the duodenum ($P = 0.028$), jejunum ($P = 0.024$), and ileum ($P = 0.049$) were greater in MCE than CTL. In conclusion, supplementation of MCE improved intestinal morphology indicating an increased absorptive capacity in calves.

Key Words: villus height, calves, crypt depth

345 Effects of an isoquinoline alkaloids-based product on performance milk yield and components of lactating dairy cows. C. Audonnet*[1], N. Cebron[2], J. Poyedessus[2], L. Leleu[2], H.

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Improving animal health and welfare with no chemical input while achieving high milk yield is a difficult task in modern dairy production systems. To this end, many solutions have been tested with uncertain results. Plant-derived products have been favorably used in several species, but scarce data are available on their effects on dairy cattle performance. The purpose of this study was to evaluate the effects of a plant-based product derived from *Macleaya cordata* containing standardized concentrations of isoquinoline alkaloids (IQs) on performance and udder health of lactating dairy cows. Primiparous (PRIM; n = 84) and multiparous (MULT; n = 602) cows from 18 commercials herds in France were evaluated. Cows were matched by lactation number, previous lactation milk yield and SCC (MULT) within farms, and were randomly allocated to receive 2 boluses containing IQs (IQS; n = 343) or to serve as a control (CON; n = 343). Boluses were designed to release an equivalent of 37.5 mg/d of IQs for 60 d, and 18.75 mg/d of IQs thereafter for 3 mo following administration. Boluses were administered during the close-up period, approximately 21 d before the expected date of calving. Data (milk yield, fat and protein, urea, SCC) from the first 5 official milk test-days were collected and analyzed retrospectively. Statistical analyses were performed using R software (v3.5.1). Results show that raw and energy-corrected milk yields were greater (two-way ANOVA, $P < 0.05$) in IQS cows with a mean increase over than 1.5 kg. No difference in milk fat, protein, and urea concentrations were observed. Overall, mean SCC was lower (50×10^3 cells/mL; Two-way ANOVA, $P < 0.05$), and the prevalence of cows with subclinical mastitis (SCC $\geq 250 \times 10^3$cells/mL) was lower ($P < 0.05$) in the IQS group on milk test-days during the first 4 mo of lactation. These results show that administration of a plant-based product with standardized concentrations of IQs during the transition period and early lactation can be regarded as a strategy to improve productivity and to reduce SCC in dairy cows.

Key Words: transition, isoquinoline alkaloids, performance

346 Effects of rumen protected choline supplementation during electric heat blanket induced heat stress. H. T. Holdorf*[1], K. A. Estes[2], B. A. Barton[2], and H. M. White[1], [1]*University of Wisconsin-Madison*, *Madison, WI*, [2]*Balchem Corporation*, *New Hampton, NY*.

The negative impacts of heat stress (HS) on health and production are partially explained by reduced dry matter intake (DMI) and the immune response (IR) to lipopolysaccharide (LPS). The objective of this study was to determine if supplementation of rumen protected (RP) choline would ameliorate the negative impacts of HS using an electric heat blanket HS model that included a 3-d baseline and 7 d of HS. Multiparous Holstein cows were blocked by milk production, and randomly assigned to a control diet (CTL, n = 7), or CTL top-dressed with RP choline (60 g; ReaShure, Balchem Corp., New Hampton, NY) during the baseline and HS (RPC+, n = 8) periods or only during the HS (RPC, n = 8) period. Daily DMI and milk production were measured, and milk components were measured by sampling the last 4 milkings of each period. Blood samples were collected from the tail vessels and BW was measured on d1 and d11. Data were analyzed using the MIXED procedure of SAS 9.4. Preplanned contrasts (PPC) were CTL vs RPC, CTL vs RPC+, CTL vs both, and RPC vs RPC+. Percent change ((d11 - d1) / d1) of BW and blood metabolites were compared with 0% change by *t*-test. Means were considered significantly different if $P \leq 0.05$, and a tendency if $0.05 < P \leq 0.1$. RP choline increased milk fat (PPC: $P = 0.05$) and tended to increase energy-corrected milk (ECM; PPC: $P = 0.08$) yields during HS. HS increased serum triglyceride concentration (+45.36%; *t*-test: $P = 0.02$) in CTL cows. HS decreased tumor necrosis factor α and alanine aminotransferase concentrations (*t*-test: $P \leq 0.01$) in all cows. The effect of HS on LPS binding protein (LBP) concentration differed (PPC: $P = 0.03$) when comparing CTL (+24%) vs RPC (+1%) and RPC+ (−17%). RP choline was able to partially mitigate the effect of HS on ECM, with no evidence for increased DMI, suggesting altered nutrient use for energy output in milk. RP choline may have modulated the IR to HS, evidenced by the differential change in LBP. The mechanism by which RP choline influences the HS induced IR should be further explored as a potential nutritional strategy to mitigate negative impacts of HS on health and production.

Key Words: Heat abatement, immune response

347 Effects of an immunomodulatory feed additive on performance of transition cows. C. S. Takiya*[1], L. K. Mamedova[1,2], M. Garcia[3], D. E. Nuzback[3], and B. J. Bradford[1,2], [1]*Kansas State University*, *Manhattan, KS*, [2]*Michigan State University*, *East Lansing, MI*, [3]*Phibro Animal Health*, *Teaneck, NJ*.

The transition period is characterized by metabolic and hormonal changes to promote milk yield at the expense of body nutrient reserves. OmniGen-AF (OMN, Phibro Animal Health, Teaneck, NJ) is a commercial feed additive that has demonstrated positive impacts on productivity and metabolism of transition cows during stressful events such as heat stress. Thirty-four multiparous cows were blocked by expected calving date and assigned to control (305ME: 14,926 kg, 1.73 parities) or OMN (56 g/d top-dressed; 305ME: 14,325 kg, 1.46 parities) from dry-off (−67 ± 3.0 d relative to actual calving date) until 49 d in milk. Dry matter intake (DMI) was measured daily from −30 to 49 d relative to parturition (RTP) and milk yield was recorded throughout the study. Blood samples were collected at enrollment, d −30, −14, −7, 1, 14, 28, and 42 RTP for assessment of plasma concentrations of glucose, free fatty acids (FFA), and β-hydroxybutyrate (BHB). Temperature-humidity index (THI) was measured throughout the study with data loggers. Models assessed fixed effects of 305ME, treatment, time, and their interaction, and random effects of cow and block; fixed effects of parity group, THI, and all interactions were also tested and retained if $P < 0.15$. Significance was declared at $P < 0.05$. Postpartum data from 6 cows were not considered in analysis due to illness. Treatment did not affect DMI either prepartum or postpartum. Cows fed OMN had lesser milk yields compared with CON (51.8 vs. 53.3 ± 1.19 kg/d). However, when peripartum THI was greater than 66, OMN cows produced more milk than CON cows (THI^2 × treatment $P < 0.01$). Treatments did not affect plasma glucose concentration either in pre- or postpartum periods. OMN decreased plasma FFA (482 vs. 331 ± 52 μ*M*) and BHB (562 vs. 464 ± 43 μ*M*) concentrations during the postpartum period. Feeding OMN from dry-off through 49 d in milk apparently slowed adipose tissue mobilization and modulated milk yield depending on environmental conditions.

Key Words: OmniGen-AF, heat stress, milk

348 Effects of non-toxigenic clostridia oral supplementation on production, health, and metabolism of Holstein cows. F. Cardoso*[1], T. Rehberger[2], J. Thompson[2], A. Smith[2], M. Griffin[2], and F. Cardoso[1], [1]*University of Illinois*, *Urbana, IL*, [2]*Arm & Hammer Animal and Food Production*, *Waukesha, WI*.

There is scarce evidence on the effects of clostridia contaminated TMR on the performance of dairy cows. Forty-one multiparous Holstein cows DIM (192 ± 111) were randomly assigned to 1 of 4 dietary treatments. The treatments were based on the clostridia load supplementation. Cows received the treatments by top-dress and oral bolus. Treatments were: CON (no added clostridia), LOW (669 ± 515 cfu/g of TMR), MED (2953 ± 2272 cfu/g of TMR) and HIG (7179 ± 5151 cfu/g of TMR). Cows were balanced with regard to parity, body condition score, and previous lactation milk yield. Observations for each variable during the adaptation phase (phase 1; cows consumed a basal diet without receiving treatment for one week) were used as a covariate for the corresponding treatment and statistical inference phase (phase 2; wk 1 through 9). A subset of cows (n = 15) were rumen-cannulated and used for rumen papillae (RP) biopsy on the last day of wk 9. Milk and dry matter intake (DMI) were obtained daily. Fecal pH was measured every 2 weeks. Cows in LOW tended to have increased ($P = 0.07$) DMI than cows in MED (24.9 and 22.5 kg/d, respectively). There was no difference ($P = 0.18$) in DMI as a percentage of BW among treatments. There was no treatment effect for milk yield ($P = 0.78$). Cows in CON had higher ($P = 0.05$) fecal pH than cows in LOW and MED (6.67vs6.57 and 6.54, respectively). Cows in CON had greater ($P = 0.01$) RP gene expression of toll like receptor 2 (TLR2) than cows in LOW, MED and HIG (2.02*vs* 0.93, 0.74, 1.1, respectively); the expression of the gene 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) was greater ($P = 0.02$) for CON and HIG compared with LOW and MED (1.44, 1.11*vs* 0.93; 0.74, respectively) and there was a tendency ($P = 0.08$) for the RP gene expression of cluster of differentiation 14 (CD14) to be greater for MED than HIG (1.16*vs* 0.66, respectively). In conclusion, cows in MED tended to have decreased DMI compared with cows in LOW. Additionally, based on RP gene expression, cows in CON had improved RP integrity than cows challenged with clostridia

Key Words: *Clostridium*, TMR, gene expression

Ruminant Nutrition: Protein/Amino Acids

349 Effects of rumen undegradable protein and amino acid sources and replacing either forage or non-forage fiber sources in postpartum cows on body composition. A. W. Tebbe* and W. P. Weiss, *Ohio Agricultural Research and Development Center, The Ohio State University*, *Wooster, OH*.

Fresh cows experience negative protein balance and mobilize body protein to meet lactational demands. Reducing mobilization by increasing metabolizable protein (MP) supply or improving the AA profile may improve milk production later into lactation. Our objective was to determine whether increasing MP concentrations at the expense of either non-forage or forage NDF (fNDF), or improving the AA profile affected empty body (EB) crude protein (CP) through transition and into early lactation. In a randomized block design, 40 primigravid and 40 multigravid Holsteins were blocked by calving date and fed a common, prefresh diet (11.5% CP). After calving to 25 d in milk (DIM), cows were fed 1 of 4 diets: 1) deficient in MP (DMP, 17% CP, 24% fNDF), 2) adequate in MP using high inclusion of lignosulfonate-treated soybean meal (AMP, 20% CP, 24% fNDF), 3) adequate in MP using a blend of RUP and rumen- protected AA (Blend, 20% CP, 24% fNDF), or 4) a similar diet but substituting forage NDF rather than non-forage NDF with the blend (Blend-fNDF, 20% CP, 19% fNDF). Cows were fed a common diet (17% CP) from 26 to 50 DIM. At 7 d before anticipated calving, and at 7, 25 and 50 DIM, body composition was estimated using the urea dilution method. Mixed models with fixed effects of treatment, day (repeated), parity, their interactions, covariate effect of prepartum measurements and random effects of block nested within parity were used to analyze data. Day by treatment ($P < 0.03$) interactions were observed for EB CP. From 7 d before calving to 7 DIM, the loss of EB CP was similar (avg. 34 g/d), but from 7 to 25 DIM, cows fed AMP (139 g/d) and Blend-fNDF (147 g/d) lost more EB CP compared with Blend (8 g/d). After treatments ended (>25 DIM), gains/losses of EB CP were similar, and the average (2 g/d) was not different from zero ($P > 0.15$). Final EB CP was similar (avg. 81.5 kg) at 50 DIM. Overall, feeding fresh cows a high MP diet from a blend of proteins and AA reduced the loss of EB CP, which may be why these cows had higher milk production during the carry-over period.

350 Effect of dietary crude protein level on lactation performance, feed efficiency, and nitrogen use efficiency of dairy cows at different stages of lactation. P. Letelier*[1], G. I. Zanton[2], and M. A. Wattiaux[1], [1]*University of Wisconsin-Madison*, *Madison, WI*, [2]*USDA-ARS, U.S. Dairy Forage Research Center*, *Madison, WI*.

Responses of dairy cows to level of dietary crude protein (CP) may depend on stage of lactation. In this study, we used a randomized complete block design to determine the effects of 4 levels of dietary CP on dry matter intake (DMI), fat- and protein-corrected milk (FPCM), feed efficiency (FE: FPCM/DMI) and nitrogen use efficiency (NUE: 100 x milk protein N/N intake) of 64 multiparous Holstein cows blocked by days in milk (DIM; initial average ± SD 71 ± 14.2 (E), 105 ± 9.6 (ME), 153 ± 21.6 (ML), and 225 ± 10.9 (L)). Diets were formulated for 13.5, 15.0, 16.5, and 18.0% CP (dry matter basis) by exchanging solvent and expeller soybean meal for soyhulls and dried ground corn. For 14 d cows were individually fed a covariate diet followed by 56 d of treatment diets. With advancing DIM, both DMI and FPCM decreased linearly (from 28.8 to 26.9 kg/d for DMI and from 44.6 to 31.7 for FPCM for E and L cows, respectively). In contrast, the responses to decreasing dietary CP were both linear and quadratic with the greatest values observed when cows were fed the 16.5% CP diet (29.2 and 41.8 kg/d for DMI and FPCM, respectively) and the lowest values observed when cows were fed the 13.5% CP diet (26.6 and 37.1 kg/d for DMI and FPCM, respectively). However, both DMI and FPCM responses were influenced by an interaction between dietary CP and DIM. In E, ME, and ML, FPCM was not different when cows were fed the 16.5 compared with the 18% CP diets (averaging 43.7 kg/d). Conversely, in L, FPCM decreased by 7.0 kg/d from 35.1 to 28.1 kg/d when CP increased from 16.5 to 18.0%. In addition, when cows were fed the 13.5% CP diet, E cows produced 9.0 kg more FPCM compared with L cows (40.8 vs. 31.7 kg/d), whereas cows fed the 18% CP diet produced 19.3 kg more FPCM in E compared with L (47.4 vs. 28.1 kg/d). Feed efficiency (1.4 ± 0.1) was not influenced by dietary CP level, but declined linearly from 1.6 to 1.2 for E to L cows. Finally, NUE declined linearly from 29.0 to 25.8% for E to L cows, and from 31.7 to 24.2% for 13.5 to 18.0% dietary CP.

Key Words: crude protein, stage of lactation, nitrogen use efficiency

351 Assessing essential amino acid availability from microbial and rumen undegraded protein in lactating dairy cows. X. Huang*[1], P. Yoder[1,2], L. Campos[1], E. Huang[1], and M. Hanigan[1], [1]*Virginia Tech*, *Blacksburg, VA*, [2]*Perdue AgriBusiness LLC*, *Salisbury, MD*.

The objective of this study was to assess a stable isotope technique for determination of essential AA availability from microbial protein in response to starch and rumen degraded protein. The study was designed as a 2 × 2 factorial arrangement with a repeated 4 × 4 Latin square design. Factors were high and low rumen degraded protein, and high and low starch. Twelve lactating cows were blocked into 3 groups according to days in milk, and randomly assigned to the 4 treatment sequences. Each period was 14 d in length with 10 d of adaption followed by 4 d of ruminal infusions of ^{15}N labeled ammonium sulfate. On the last day of each period, a ^{13}C-labeled AA mixture was infused into the jugular vein over a 6 h period to assess total AA entry. Rumen, blood, and urine samples were collected during the infusions. Ruminal microbes and blood samples were assessed for AA enrichment. Total plasma AA absorption rates were derived for each essential AA from the plasma, ^{13}C-AA enrichment. Essential AA absorption from microbial protein was calculated from total AA absorption based on ^{15}N enrichment in blood and rumen microbes. Microbial AA absorption rates derived from the isotope dilution model were greater for the high rumen degraded protein diets and unaffected by starch level, except for Met, which decreased with high starch. Microbial protein outflow estimated from purine derivatives increased with rumen degraded protein and was not significantly affected by starch, which was consistent with estimates from the isotope dilution model. Total AA absorption rates derived from the isotope dilution model were similar to estimates from CNCPS (v6.5), but a lower proportion of absorbed AA was derived from microbial protein for the former method. Assuming 7.6% loss during first pass through the splanchnic tissues, the average essential AA digestibility for microbial AA was 82%, which varied across AA and by treatment. The new method has the advantage of quantifying the essential AA availability for individual EAA from rumen undegraded protein and microbial protein.

Key Words: dairy cow, microbial protein, amino acid availability

352 Dietary energy source and rumen-protected amino acids: Effects on milk production and plasma amino acid concentrations in dairy cows. Y. Zang*[1], L. H. P. Silva[1], Y. Geng[2], M. J. Lange[3], N. Q. Dattolico[1], N. L. Whitehouse[1], M. Miura[4], M. A. Zambom[3], and A. F. Brito[1], [1]*University of New Hampshire*, *Durham, NH*, [2]*Chinese Academy of Agricultural Sciences*, *Beijing, China*, [3]*Universidade Estadual do Oeste do Paraná*, *Marechal Cândido Rondon, Brazil*, [4]*Ajinomoto Co. Inc*, *Kawasaki-shi, Japan*.

Our previous study demonstrated that reduced dietary starch level limited milk and milk protein yield in dairy cows receiving MP-deficient diets. Supplementation of reduced starch diets with fat could be a feeding strategy to improve milk production. We aimed to investigate the interactions between energy source (starch vs. fat) and rumen-protected (RP) Met, Lys, and His (MLH) on performance and plasma AA in dairy cows fed MP-de-

Table 1 (Abstr. 353). Plasma AA concentrations (µ*M*) for cows fed RP-AA supplements and associated statistics

Item	CON	INF	RPL+ SMM	SML	SEM	*P*-value
Lysine	81.0^{c}	120.7^{a}	103.9^{b}	118.8^{a}	5.18	<0.001
Methionine	27.6^{b}	43.9^{a}	41.2^{a}	42.6^{a}	2.02	<0.001
Cystine	20.8	21.2	20.9	20.9	0.70	0.38
Cystathionine/Allocystathionine	2.2^{b}	2.8^{a}	2.8^{a}	2.8^{a}	0.22	0.04
Homocystine	2.5	2.6	2.5	2.5	0.15	0.90
Taurine	51.5	56.2	57.5	57.5	4.1	0.61
TSAA	104.6^{b}	126.7^{a}	124.9^{a}	126.2^{a}	5.1	0.005
Total AA	2272	2305	2331	2365	53.3	0.17
Metabolizable Lys, g/kg of product[1]	—	—	184	355	—	—
Metabolizable Met, g/kg of product[2]	—	—	616	122	—	—

[a–c]Means within rows differ at $P < 0.05$.

[1] Analyzed Lys content of products: RPL 39.4% and SML 44.1%.

[2] Analyzed Met content of products: SMM 73.8% and SML 15.0%.

ficient diets. Sixteen multiparous Holstein cows (112 ± 28 DIM) were used in a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments. Each period lasted 21 d with 14 d for diet adaptation and 7 d for sample collection. Treatments included high starch (HS), HS + RPMLH, reduced starch + RP-fat (RSF), and RSF + RPMLH. Basal diets consisted of (DM basis) 50% forage and 50% concentrate. The HS diet contained (DM basis) 26% ground corn, while the RSF diet had 16% ground corn replaced with 15% soyhulls and 1.5% RP-fat (i.e., palmitic acid-enriched supplement). Dietary NE_L, starch, and CP averaged 1.53 Mcal/kg, 32.6% and 15.9% for HS diets, and 1.59 Mcal/kg, 21.7% and 16.8% for RSF diets, respectively. Smartamine M, Aji-Pro L, and an Ajinomoto prototype RP-His product were fed. Data were analyzed with the MIXED procedure of SAS. Dietary treatments had no effects on DMI and milk yield. However, feeding RSF diets enhanced feed efficiency (1.57 vs. 1.54) and milk fat yield (1.65 vs. 1.50 kg/d) compared with HS diets. Concentration of milk fat increased but that of milk true protein decreased with RSF vs. HS diets. Both MUN and PUN were greater and milk N efficiency (30.6 vs. 29.1%) was lower for RSF vs. HS diets. Supplemental RPMLH tended to improve milk true protein concentration. No significant interactions between RPMLH and energy source were observed for plasma AA. Additionally, RSF diets elevated plasma concentrations of Arg, Ile, Thr, and Ala but reduced Leu relative to HS diets. Plasma Met and His increased with RPMLH. In brief, substitution of ground corn with soyhulls and RP-fat improved feed efficiency and milk fat yield but appeared to reduce N utilization.

Key Words: amino acid, dairy cow, energy source

353 Using the plasma amino acid dose-response method to validate the bioavailability of methionine and lysine in Smartamine ML.

N. L. Whitehouse*[1], S. M. Hollister[1], L. H. P. Silva[1], and K. A. McLain[2], [1]*University of New Hampshire*, *Durham, NH*, [2]*University of Nebraska-Lincoln*, *Lincoln, NE*.

Rumen protected sources of Met and Lys need to be held to the same scrutiny as any ingredient in their capacity to provide metabolizable Lys and Met. The primary objective of this trial was to determine the Lys and Met bioavailability of Smartamine ML using the plasma AA dose-response method. Eight multiparous Holstein cows (159 ± 33 DIM) fitted with ruminal cannulas were used in a replicated 4 × 4 Latin square with 7-d periods. Treatments were (g/d): 1) 0 (CON), 2) 60 Lys + 20.5 Met abomasally infused (INF), 3) 60 Lys + 20.5 Met from Smartamine ML (SML; Adisseo), and 4) 60 Lys from a rumen-protected Lys supplement + 20.5 DL-Met from Smartamine M (RPL+SMM). Intake and milk yield were measured daily. Blood was collected from tail vein 2, 4, 6 and 8 h after the 0500h feeding the last 3 d of each period. Data were analyzed using PROC MIXED and REG procedures of SAS. Significance was declared at $P \leq 0.05$. Milk yield (38.5 kg/d) and DMI (26.2 kg/d) were not affected by treatment. Plasma Lys, Met and total sulfur AA (TSAA) concentrations were increased for the 3 AA treatments compared with CON. Plasma Lys was higher for INF and SML than for RPL+SMM. Based on response slope differences using plasma Lys expressed as a % of TAA-Lys, RPL+SMM and SML were 46.7% (±4.8) and 80.4% (±4.8) as effective as INF in providing metabolizable Lys. Using plasma TSAA expressed as a % of TAA-TSAA, RPL+SMM and SML were 83.5% (±5.0) and 81.3 (±3.3) as effective as INF in providing metabolizable Met.

Key Words: lysine, methionine, plasma

Animal Health: Mastitis 1

354 Evaluation of the cost of mastitis by *Streptococcus uberis* and the profitability of vaccination protocols. A. M. Mesa and S. Calsamiglia*, *Animal Nutrition and Welfare Service, Univeristat Autonoma de Barcelona, Bellaterra, Spain.*

The objective of the research was to evaluate the cost of *S. uberis* mastitis and the profitability of vaccination protocols. Literature data were reviewed to determine the average incidence and effects of clinical and subclinical *S. uberis* mastitis and the impact of vaccination on the incidence and effects. A stochastic dairy herd simulation program (www.dairyfarm.es) was used to simulated different scenarios. The modeled farm was a 300 adult cows producing 11.500L/cow/year with an SCC of 200 × 10^3 cells/mL. Milk price was 0.35€/L with 0.02€/L penalty if somatic cell count were above 300 × 10^3/mL. The behavior of *S. uberis* mastitis is variable and may become chronic in some cases. However, average values of literature were used. Control scenario was tested with an average incidence of 12.5%, where 4% were clinical and 8.4% subclinical mastitis. In all cases, the incidence in primiparous cows was 25% of that of multiparous cows, culling risk was 36% and the risk of recurrence increased 35% after the first case. Clinical cases lasted 13 d where milk production was reduced 5% daily, conception rate was reduced by 26% during 42 d after the infection, and somatic cell count was multiplied by 4. Treatment cost was 30€/case including antibiotics and labor, with a recovery rate of 90%, and milk had to be withdrawn for 10 d. Subclinical cases lasted 45 d with a 7% daily reduction in milk yield and somatic cell count was multiplied by 4, but with no effects on reproduction. The vaccine (UBAC, Laboratorios Hipra SA, Spain) was applied 3x/year at 4,7€/dose including vaccine cost and labor, and reduced the incidence of clinical and subclinical diseases by 50%. In cows vaccinated but infected, the effects were reduced by 50%. All simulations were conducted for 5 years and were repeated 66 times. The cost of a case of mastitis by *S. uberis* was on average 88.80€. With an average incidence of 12.5%, vaccination increased the gross margin by 47€/cow/yr. The sensitivity analysis indicates that the breakeven point for the application of the vaccine is for an incidence of 3.4%.

Key Words: *S. uberis* mastitis cost, vaccination profitability, dairy herd economic model

355 Molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) and associated risk factors with the occurrence of goat mastitis. M. Ijaz*, M. Altaf, M. Kashif Iqbal, A. Rehman, M. Avais, and A. Ghaffar, *University of Veterinary & Animal Sciences, Lahore, Punjab, Pakistan.*

The current study was designed to determine the prevalence of *Staphylococcus aureus* (*S. aureus*) related subclinical mastitis (SCM) and detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy goats in Pakistan. A total of 385 milk samples were collected from Mianwali (n = 192) and Narowal (n = 193) districts and initially screened for high SCC by using Surf Field Mastitis Test (SFMT). SFMT based positive samples which shows clumping were further processed for the isolation and identification of *S. aureus* by using mannitol salt agar. The positive samples were subjected to disc diffusion test using oxacillin discs and further confirmed through amplification of *mecA* gene to detect resistance against Methicillin in *S. aureus* isolates. Hypothesized risk factors for the occurrence of SCM were recorded and analyzed through logistic regression model in SPSS version 20.0. Initially variables were analyzed by univariable analysis. At significance level of $P < 0.2$, the variables were tested. Then these variables were included in multivariable logistic regression model. The study revealed 39.2% (151/385) prevalence of SCM by SFMT which was mainly 80.8% (122/151) caused by *S. aureus*. MRSA prevalence through disc diffusion test was 18.8% (23/122) while PCR based prevalence was 6.5% (8/122). All the study isolates showed 99% homology with MRSA isolates of India, Turkey and Japan with accession numbers MH798869, EU790488 and NG047938 respectively, available in NCBI database. Milker's care and hygienic measures during milking, milk yield, use of teat dips, presence of ticks, mixed type of grazing and services by professionals were key risk factors associated with the occurrence of SCM in goats. This is the first report regarding the molecular characterization of MRSA isolated from dairy goats in Pakistan and the study will be helpful to provide information for developing control strategies against mastitis in goats.

Key Words: subclinical mastitis, dairy goats, methicillin-resistant *Staphylococcus aureus*

356 Assessing the risk of subclinical intramammary infection in non-clinical quarter(s) by test day somatic cell count or clinical symptoms. K. Morrill* and J. Scillieri Smith, *Cornell University, Ithaca, NY.*

The objectives of this project were 1) to evaluate the rate of subclinical mastitis (SCM) in non-clinical quarters (NCQ) of cows with clinical mastitis (CM), cows with low somatic cell count (SCC; LSCC; < 200,000 cells/mL) and high SCC (HSCC; > 200,000 cells/mL) and 2) evaluate the rate of persistent infection. Herds (n = 4) aseptically sampled milk from quarters of cows identified with CM and herdmates with LSCC or HSCC, based on DHIA testing and confirmed upon sampling. Samples were cultured using standard microbiological methods by Quality Milk Production Services (Canton, NY). Quarter level SCC was analyzed by an optic cell counter (DeLaval Cell Counter, Tumba, Sweden). All quarters with intramammary infections (IMI) were re-sampled 2–4 weeks later for aerobic culture and SCC to determine persistency of infection. Data were analyzed using SAS 9.4. A total of 1698 quarters (CM = 246, NCQ = 575, LSCC = 486, HSCC = 391) from 433 cows were included in the study. Overall, 25.7% of samples had an IMI at initial culture. Mastitis pathogens were identified in 55.3% of CM quarters and in 24.6% of NCQ. Samples from NCQ and HSCC cows were at a greater risk of having an IMI as compared with samples from LSCC cows (OR = 2.7207; CI = 1.9833 – 3.7322). One hundred and 2 CM cows (41.46%) had an IMI in at least one NCQ. Only 26 of the 209 CM cows had an IMI in the CM quarter and at least one NCQ concurrently. Only 10 CM cows had an IMI in all 4 quarters, while 49 CM cows did not have an IMI in any quarter. Follow-up samples from CM quarters were at a lower risk of having a IMI (OR = 0.519; CI = 0.3042 – 0.8281) as compared with NCQ, LSCC and HSCC quarters. Overall, 8% of quarters had a persistent IMI. High SCC quarters had a higher risk of having a IMI at follow-up sampling as compared with all other sample groups (OR = 2.9455; CI = 1.6937 – 5.1224). In conclusion, samples from CM quarters were at a greater risk of having an IMI as compared with NCQ, HSCC and LSCC quarters, however, NCQ and quarters from a HSCC cow were at a greater risk of having an IMI as compared with samples from LSCC cows.

Key Words: mastitis, somatic cell count, subclinical

357 Partial budget analysis of selective dry cow therapy strategies. A. K. Vasquez[1], S. M. Rowe*[2], S. M. Godden[2], P. J. Gorden[3], A. Lago[4], E. Royster[2], J. Timmerman[2], M. J. Thomas[5], R. A. Lynch[1], and D. V. Nydam[1], [1]*Cornell University, Ithaca, NY*, [2]*University of Minnesota, St. Paul, MN*, [3]*Iowa State University, Ames, IA*, [4]*Dairy Experts, Tulare, CA*, [5]*Dairy Health & Management Services, Lowville, NY.*

This study conducted partial budget analysis using MonteCarlo simulation with @Risk software to estimate the net economic impact for herds that switch from blanket dry cow therapy (BDCT) to culture- or algorithm-guided selective dry cow therapy (SDCT). Initial models assumed no differences in health outcomes between strategies, as was concluded in a previous trial by the authors. 10,000 iterations using expenditures associated with dry-off procedures and health outcomes during 1–30 DIM were used to model net economic herd-level impacts. Fixed values were insert-

ed for variables expected to have minimal variation on US dairies and appropriate distributions were used for components that were assumed to vary. Costs for fixed or variable inputs and distribution types were derived from previously published models, current industry databases, or from professional experience. For culture-guided SDCT, on average, producers could expect to save \$2.14 per cow-dry-off as compared with BDCT. For algorithm-guided SDCT, the mean net impact was \$7.85. Seventy-six and 100% of iterations had a net impact ≥ \$0 for culture- and algorithm-guided SDCT, respectively, indicating that the strategies were profitable in most of the herd conditions evaluated. The largest contributors to variance (~75% and ~25%) in both models were the percent of quarters treated at dry-off and the cost of antibiotics. Next, we investigated the economic impact in situations where SDCT increased mastitis cases during the next lactation. For this sensitivity analysis, subclinical and clinical mastitis were both set to increase by 1%, 2%, or 5% and net economic impacts were determined at fixed values of 20, 40, 60 and 80% antibiotic use at dry-off. Analysis indicated that if a 1% or 2% increase in mastitis resulted, in many situations SDCT continued to have net benefits over BDCT, particularly when use of these strategies resulted in lower levels of antibiotic use for the herds. At each level of evaluated antibiotic use, when mastitis was increased by 5%, mean economic impacts were negative for 95% of iterations. This partial budget analysis found that in the majority of situations, 2 SDCT strategies produce a positive net economic impact over BDCT.

358 Effect of treatment at dry-off with intramammary antibiotics, internal teat sealants, or both on milk production in dairy cows.

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Milk production is the main source of income to the dairy industry and mastitis remains the greatest challenge for dairy producers. Among the approaches implemented to control and prevent mastitis on dairies are vaccination, pre and post milking teat dip and treatments at dry off including intramammary antibiotics and teat sealants. The objectives of our study were to evaluate the effect of different treatments at dry off on milk production and somatic cell count (SCC) in the subsequent lactation. A block randomized trial was conducted between December 2016 and February 2018 on 8 herds from 4 of the top 10 milk producing counties in California: Tulare, Kings, Stanislaus, and San Joaquin. Cows were enrolled during winter and summer seasons to account for the seasonal variability and eligible cows were treated at dry off with either intramammary antibiotics (AB), internal teat sealant (ITS), both or none and followed through 150 d in milk (DIM) post calving. Milk production and SCC data were extracted from monthly milk test day records (Dairy Comp305, Valley Ag Software, Tulare, CA). Two-piece spline linear mixed models were used to model the milk production (kg) and $\log_{10}$ of SCC (cells/mL). After accounting for parity, breed, season and dry period duration, the milk model showed a significant increase in milk production (1.88 kg/d) in cows treated with both AB and ITS at dry off in comparison to the controls; while there was a numerical increase in milk produced by cows that received either AB or ITS. Different dry cow treatments were associated with a significant reduction in the $\log_{10}$ SCC during the first 150 d following calving. The greatest reduction was associated with administration of both AB and ITS (−0.41; $P < 0.01$), followed by AB (−0.30; $P < 0.01$), and finally ITS (−0.19; $P = 0.03$) in comparison to controls. Dry cow treatments can be used selectively to address specific herd production and milk quality goals. Dairies with high SCC may benefit from treating cows at dry off with both AB and ITS .

Key Words: dry cow treatment, somatic cell count, clinical trial

Breeding and Genetics

359 Comprehensive analyses of 723 transcriptomes enhance genetic and biological interpretations for complex traits in cattle. G. E. Liu*, *Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD.*

To systemically establish connections at RNA level between tissue/cell types and complex traits in cattle, we uniformly analyzed 723 RNA-seq data from 91 tissues and cell types. We built a comprehensive gene atlas and studied tissue specificity of genes. We demonstrated that tissue-specific genes significantly reflected the tissue-relevant biology, showing distinct promoter methylation and evolution pattern (e.g., brain-specific genes evolve slowest, while testis-specific genes evolve fastest). Through integrative analyses of those tissue-specific genes with large-scale genome-wide association studies, we detected relevant tissues/cell types and candidate genes for 45 economically important traits in cattle, including blood/immune system (e.g., *CCDC88C*) for male fertility, brain (e.g., *TRIM46* and *RAB6A*) for milk production, and multiple growth-related tissues (e.g., *FGF6* and *CCND2*) for body conformation. We validated these findings by using epigenomic data across major somatic tissues and sperm. Collectively, our findings provided novel insights into the genetic and biological mechanisms underlying complex traits in cattle, and our transcriptome atlas can serve as a primary source for biological interpretation, functional validation, studies of adaptive evolution, and genomic improvement in livestock.

Key Words: cattle gene atlas, GWAS, RNA-seq

360 Imputation and investigation of sequence genotypes for 6,735,530 variants of 39,048 Holsteins. A. Al-Khudhair*[1], J. R. O'Connell[2], D. J. Null[1], and P. M. VanRaden[1], [1]*USDA/Animal Genomics and Improvement Laboratory*, *Beltsville, MD*, [2]*The University of Maryland School of Medicine*, *Baltimore, MD.*

Previous US studies of Holstein genotypes from run5 of the 1000 Bull Genomes Project used sequence variants in exons and very close to genes, whereas current study of run7 genotypes also includes intronic and intergenic loci. After data cleaning/editing, sequence genotypes for 6,735,530 variants of 917 Holsteins were selected from run7 raw data, in addition to array genotypes from the Council on Dairy Cattle Breeding (Bowie, MD) database, which included either 79,294 SNP from routine predictions or 643,059 SNP from imputed high-density (HD) genotypes using Findhap, version 3. A total of 39,048 Holstein bulls had either sequence or imputed HD genotypes, and all were imputed to sequence. Editing and imputation tests combining sequence and HD array genotypes revealed higher genotype error rate with run7 genotypes than from previous run5 genotypes. Genome-wide association was performed with deregressed milk and fat phenotypes of Holstein bulls using a mixed model framework. That framework included an intercept and a polygenic random effect estimated with a genetic relationship matrix constructed from 79,294 markers from the imputed genotype file for December 2019 US genomic evaluations. Residual error was modeled using a diagonal matrix with deregressed animal-specific reliabilities. Milk and fat had 488 and 603 markers, respectively, with a P-value of < 1E-10. Known major loci, such as in DGAT1, ABCG2, and β-casein, had highest effects in official predictions, on the contrary, nearby linked loci had higher effects in imputed HD or imputed sequence data. This indicates that using more variants does not ensure localizing causal variants; however, official predictions included about 800,000 genotyped and phenotyped cows that were not included in the HD or sequence studies. Phenotypic effects were also estimated by multiple regression for 13 traits, but convergence was incomplete. Annotation of results and conditional analyses is underway to investigate if intronic and intergenic loci also directly affect phenotypes of interest and to identify additional candidate loci to be included in future genotyping chips.

Key Words: genome-wide association, genotype, sequence imputation

361 Genomic prediction with single-step genomic BLUP using a subset of genotypes in US Holstein. Y. Masuda*, S. Tsuruta, and I. Misztal, *University of Georgia*, *Athens, GA*.

As of January 2020, the US dairy database includes more than 3.8 million genotypes. Most of the genotypes are for heifers, and only a fraction of them have phenotypes. Although the use of all genotypes in genomic prediction is an ideal strategy, the same prediction-accuracy can be obtained using a subset of genotypes with a decrease of computing cost in single-step genomic BLUP (ssGBLUP). The objective of this study was to compare genomic predictions of young bulls between all genotypes and a subset of genotypes in US Holstein. We calculated the benchmarks using the full data set, provided by the Council on Dairy Cattle Breeding, including 61M phenotypes of 305-d protein yield, 36M pedigrees, and 2.3M genotypes. The benchmarks included daughter-yield-deviation (DYD) from pedigree BLUP, DYD from ssGBLUP, and GPTA from ssGBLUP. We cut off the last 4 years from the full data, and the truncated set included 841K genotypes up to 2014 (841K; ALL). Out of it, we created 2 sets of genotypes including bulls only (142K; BULL) and bulls and cows with records and with both parents known (256K; BULLCOW). For validation, we chose 3,250 bulls that had at least 50 phenotyped-daughters in 2018, but that had no daughters in 2014. The coefficient of determination (R^2) and the slope coefficient (b_1) were calculated from a linear regression of the benchmark on GPTA. For GEBV-ssGBLUP as the benchmark, R^2 was 0.82 for ALL, 0.76 for BULL, and 0.82 for BULLCOW. Whereas b_1 was around 0.90 in ALL and BULLCOW, BULL showed a lower value (0.83). We observed the same tendency in the other benchmarks. Using bull genotypes alone drop the accuracy probably because of limited information. The use of cow genotypes increases accuracy and reduces bias. The inclusion of genotyped heifers does not improve the accuracy of bull predictions. Statistics for validations based on GPTA-ssGBLUP indicate good stability of genomic predictions. Routine analyses by ssGBLUP can include only genotypes for bulls with daughters and cows, with the remaining animals predicted indirectly.

Key Words: genomic prediction, single-step GBLUP, selected genotypes

362 Accuracy of indirect predictions based on prediction error covariance from single-step genomic BLUP. D. Lourenco*[1], I. Aguilar[2], A. Legarra[3], A. Garcia[1], Y. Masuda[1], S. Tsuruta[1], and I. Misztal[1], [1]*University of Georgia*, *Athens, GA*, [2]*INIA*, *Las Brujas, Canelones, Uruguay*, [3]*INRA*, *Castanet Tolosan, France.*

One of the ways to deal with the ever-increasing number of genotyped animals in single-step genomic BLUP (ssGBLUP) evaluations may be to use only genotyped animals with complete information in the official evaluation and compute indirect predictions (IP) for the remaining young genotyped animals. However, if IP are going to be published, there is a need for a measure of accuracy that reflects the standard error of IP. This measure should be similar to the accuracy of GEBV to validate the usefulness of IP. The objective of this study was to implement formulas to compute accuracy of IP based on the prediction error covariance matrix from ssGBLUP. Using field data, complete ssGBLUP evaluations were run with up to 60k genotyped animals. Reduced ssGBLUP evaluations considered genotypes for up to 55k animals. BLUPF90 was used to compute both complete and reduced evaluations. Accuracy of GEBV in the complete evaluation was computed based on PEV, whereas in the reduced evaluation the left-hand side of the mixed model equations was stored. Using POSTGSF90, the submatrix of prediction error covariance (PEC) for GEBV of genotyped animals was extracted and converted to PEC of SNP effects. Using the same software, GEBV were converted to SNP effects. PREDF90 was used to obtain IP and accuracy of IP for up to 5k young validation animals. Accuracy of IP was computed as a function of PEC for SNP effects and genotypes. Tuning and blending parameters were accounted for by the formulas implemented in POSTGSF90 and PREDF90. Correlations between accuracy of GEBV and IP for the 5k validation animals were greater than 0.98, as well as correlations between predictions. These results show that accuracy of IP can be calculated based on ssGBLUP evaluations without the need to run an extra SNP-BLUP evaluation to obtain PEC for SNP effects. Future steps will involve the expansion of this method to large-scale ssGBLUP evaluations, where the algorithm for proven and young is used, likely based on approximations.

Key Words: genomic predictions, reliability

363 Development and validation of a medium-density chip array for bovine.

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Genomic testing has revolutionized animal breeding in almost all agricultural species. Dairy, for the last 12 years, has benefited from cost-effective high-throughput genotyping to improve selection accuracy and reduce generation interval. Here we report on the development of a new 65k Affymetrix Axiom bovine array. For continuity and accuracy of imputation, SNPs on the array have been extracted from existing variants heavily used in the US national dairy genomic evaluation. The recently published Bovine genome assembly (ARS-UCD1.2) was used when selecting the markers along with available information on major effects and known defects to enhance the content and improve the design. The array design featured about 69k SNP markers that converted into 63,836 working assays and several private SNPs. Of the carefully selected set of 60k markers recommended by The Council on Dairy Cattle Breeding (CDCB), about 55k were carried over onto the new chip to ensure high compatibility. Markers covered all autosomes, X and Y chromosomes, and were evenly spaced across the genome with average median gap of 32k bp among autosomes. All 200 ISAG markers were included in the design, and several essential markers used by CDCB to improve on the data quality control such as sex-specific markers, breed-specific markers and markers used for parental verification. Moreover, we included markers to cover all known causal mutations and targeted specific haplotype regions with previously identified associations with deleterious mutations. For validation, we genotyped 768 animals which were previously genotyped using other commercially available SNP chips and compared SNP calls and clustering quality. Finally, we developed priors for all markers on the array to provide the genotype calling algorithm information to assist with genotype calling in situations where genotype clusters are lowly represented.

Key Words: genomics, chip, bovine

Dairy Foods: Chemistry

364 Characterization of a commercial whey protein hydrolysate and its use as a binding agent in whey protein isolate agglomeration process: A preliminary study.

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Enzymatic hydrolysis is used to improve the functional characteristics of whey proteins. The type and specificity of the enzyme influence the properties of the resultant hydrolysate. In a recent invention, the whey protein hydrolysate (WPH) was utilized as a binder to facilitate the agglomeration of whey protein isolate (WPI). The first objective was to characterize the chemical properties of 3 lots of WPH obtained from a commercial manufacturer. The degree of hydrolysis (DH) of WPH was between 13.82 and 15.35% and not significantly ($P > 0.05$) different between the lots. From the MALDI-TOF, 10 to 13 and 2 different peptides were observed in the range of 2.5 – 5 and 5 – 8 kDa, respectively. It was also observed from the HPLC that the major whey proteins were completely hydrolyzed indicating a consistent hydrolysis. The second objective of the study was to evaluate the effectiveness of WPH as a binder in wet agglomeration of WPI. For this purpose, a 3 × 3 × 2 factorial design was conducted with pre-wet mass (60, 100, and 140 g), WPH concentration (15, 20, and 25%), and flow rate (11 and 16 rpm) as independent variables. Agglomeration of WPI was carried out in a top-spray fluid bed granulator (Midi-Glatt, Germany). All the experiments were performed in triplicates using 3 lots of WPH. Agglomerated WPI samples were stored at 25°C and analyzed for moisture, water activity, relative dissolution index (RDI), foaming, and emulsifying capacity. There was a significant ($P < 0.05$) difference in moisture content (range: 3 – 20%) and water activity (range: 0.08 – 0.80) among the agglomerated powders. High moisture content and water activity were observed for the treatments with higher pre-wet volume and higher flow rate and also resulted in clumping of the powders. The treatment that has 60 g of pre-wet, 20% WPH concentration and 16 rpm feeding speed combination had the highest RDI among all the samples. In conclusion, WPH can be used as a potential alternate to soy lecithin in wet agglomeration.

Key Words: agglomeration, whey, hydrolysate

365 Interaction of strawberry phenolic compounds with milk proteins.

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The bioactive properties of polyphenols have gained great importance due to the proven health benefits and incorporating it in milk system can serve as a basis for functional foods. But the question is what happens when they are present together because polyphenols have significant binding affinity to milk proteins, which can lead to the formation of protein-polyphenol complexes and significantly affect biological activities. Therefore, the present investigation is planned to study the interaction of strawberry polyphenols with milk proteins at different protein concentration (1–40 mg/mL), polyphenol concentration (0.5–2 mg/mL), processing temperature (73 C/ no hold and 90 C/15 min 120 C/15 min) and pH values (6.7 and 3.57). The reconstituted skim milk (8.5% SNF) was also prepared to have a combined effect of casein and whey proteins. The results (3 replicates) were analyzed using ANOVA and the Tukey test (P-values ≤0.05). Statistical analysis was performed using Prism software (version 5.01). It was found that binding of polyphenols was highest with casein (30mg/mL) and least with skim milk (8.5% SNF) and increased from 55 to 70% as casein concentration increased from 1 to 10 mg/mL. Antioxidant activity is measured by using DPPH assay. The DPPH activity of polyphenol remain unchanged with skim milk and decreased as protein concentration increased from 1 to 40 mg/mL. Effect of heating at different time-temperature combination had no effect on the binding of polyphenol and antioxidant activity. Increasing the pH of polyphenol from 3.57 to 6.7 resulted in increased antioxidant activity for casein, whey protein and milk-polyphenol system. Further, from spectrofluorimetric analysis of samples, it was observed that hydrophobic sites decreased from 400 to 20 at 8% SNF of milk, from 800 to 80 at 2.5% casein and from 700 to 80 at 2% whey protein from control to the samples blended with polyphenol extract. ANS (fluorescent dye 1-anilinonaphthalene-8-sulphonate) binding affinity decreased from 0.05 to 0.02 at 8% SNF, from 0.04 to 0.03 at 2.5% casein and from 0.06 to 0.05 at 2% whey protein from control to the samples blended with Polyphenol extract. These results will help in designing manufacturing processes of functional dairy products that improve yield and quality attributes.

Key Words: polyphenol, protein, interaction

366 Measurement of lactose in "lactose-free" products.

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The industrial production of "lactose-free" dairy products is typically achieved through the use of β-galactosidases to hydrolyze the lactose naturally present. This process not only generates glucose and galactose but also a range of lactose analogs and galactooligosaccharides (GOS) that are produced through concomitant transglycosylation reactions. As a result, "lactose-free" dairy products often contain a range of lactose analogs and GOS at concentrations comparable to or higher than the residual lactose content. The most prominent by-product in "lactose-free" samples is allolactose (1,6-β-D-galactosyl-glucose) which can often reach total concentrations of ~0.05 g/L when final concentration of lactose can in some cases be < 0.01 g/L. Traditional enzymatic lactose determination procedures involve the use of a β-galactosidase to hydrolyse the residual lactose in "lactose-free" products coupled with measurement of the glucose or galactose released. With these sample types, the selectivity of the β-galactosidase employed is of paramount importance since the non-selective enzymatic hydrolysis of the lactose analogs such as allolactose present in the sample also leads to additional glucose/galactose formation and results in the overestimation of lactose content. Briefly, this novel enzymatic procedure comprises a sample pre-treatment to enzymatically remove glucose, a selective b-galactosidase hydrolysis step, and a creep calculation to account for unselective hydrolysis. This method was fully characterized in terms of its linear range (2.3–113 mg lactose/100 g), limit of detection (LOD) (0.13 mg lactose/100 g), limit of quantification (LOQ) (0.44 mg lactose/100 g) and reproducibility (≤3.2% CV). A range of commercially available lactose-free samples was analyzed. The lactose values obtained compared favorably with those obtained using quantitative high-performance anion exchange chromatography – pulsed amperometric detection (HPAEC-PAD) analysis. This method achieved Official Method First Action Status at the AOAC annual meeting in September 2019.

Key Words: analytical method, lactose-free, enzymatic assay

367 Influence of ionic environment on acidified micellar casein gels.

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Recent advances in filtration have led to an increased interest in micellar casein products. These microfiltered milk products are mostly depleted of whey proteins and differ from caseinates as they are believed to retain much of the original micellar structure found in milk. While much is known about the impact of ionic strength on milk gels generally, research on whey-depleted micellar casein gels is limited. We found that the ionic environment of casein dispersions had a significant impact on acid gel properties. Micellar casein dispersions prepared in water at 4% protein exhibited low conductivity (0.9 mS cm^{-1}), indicative of low ionic strength, compared with reconstituted skim milk (4.9 mS cm^{-1}). When acidified with thermophilic starter cultures at 40°C, micellar casein dispersions made in NaCl or milk permeate gelled at lower pH and formed firmer gels (at pH 4.6) compared with samples prepared in water. 4% casein dispersions did not form a gel at pH 4.6 if prepared in NaCl at 150 mmol kg^{-1} and above. Dispersions were also prepared at 4 and 8% protein with increasing concentrations of NaCl and cold-acidified at 2°C to pH 4.6 using 0.5 M HCl. Cold-acidified dispersions were then warmed to 30°C at a rate of 0.5°C/min in a rheometer to measure gel development without gelation pH and time as confounding factors. Cold-acidified dispersions formed a gel around 25°C as long as ionic strength was below a certain threshold level, which was dependent on protein level. Storage modulus of cold-acidified casein gels increased, and loss tangent decreased, with increasing ionic strength and protein content. This research shows that weaker casein gels may be formed by lowering the ionic strength of the dispersion and the mechanisms for the weaker gel can be partially explained by the higher gelation pH but is also likely due to electrostatic and hydrophobic interactions resulting from the lower ionic strength. Very low ionic strength may encourage more hydrophobic interactions between caseins allowing for gelation at higher pH values. Control of the ionic environment of micellar casein dispersions greatly impacts its acid gelation behavior.

Key Words: casein, acid, gel

368 Modeling the effect of temperature and water activity on thermal resistance of *Salmonella* in dairy powders. X. Wei*[1], B. Chaves[1], M.-G. Danao[1], S. Agarwal[3], and J. Subbiah[2], [1]*University of Nebraska, Lincoln, NE*, [2]*University of Arkansas, Fayetteville, AR*, [3]*Mars Wrigley, Chicago, IL*.

Salmonella persistence in dairy powders has caused several foodborne outbreaks. The selection of pasteurization processing conditions requires determination of the thermal inactivation kinetics of *Salmonella* in dairy powders. The objectives of this study were to 1) determine the thermal inactivation kinetics of dairy powders at different fat content and water activity (a_w); 2) evaluate multiple models for describing the effect of temperature, a_w, and fat content on inactivation of *Salmonella* in dairy powders. Two types of dairy powders, nonfat dry milk (0.62% fat content, wt/wt) and whole milk powder (29.46% fat content) were inoculated with a 5-strain *Salmonella* cocktail and equilibrated to 3 a_w levels (0.10, 0.20 and 0.30) for the isothermal treatment at 75, 80 and 85°C to obtain the *D*- and *z*-values. Response surface and modified Bigelow models were used to fit the collected data. The thermal resistance of *Salmonella* significantly increased ($P < 0.05$) as a_w decreased, which suggested that a higher temperature or longer processing time would be required to achieve the desired inactivation of *Salmonella* at lower a_w. Fat content did not have a significant impact on thermal inactivation kinetics and therefore, data from both dairy powders were pooled to develop a combined model. Response surface model was compared with modified Bigelow model. Modified Bigelow model performed well to predict *D*-values (root-mean-square error (RMSE) = 1.47 min) and log reductions (RMSE = 0.47 log cfu/g), when compared with the response surface model (RMSE = 1.61 min and 0.48 log cfu/g). This study provides guidance to the dairy industry to understand the influence of temperature and a_w on the thermal inactivation of *Salmonella* in dairy powders and identify the proper temperature and time combinations for the development and implementation of the pasteurization process to ensure food safety.

Key Words: modified Bigelow model, response surface model, D and z values

Forages and Pastures

369 Effect of drought stress on fiber digestibility of corn for silage. G. Ferreira* and C. L. Teets, *Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

The objective of this study was to determine the effect of drought stress on ruminal in situ neutral detergent fiber digestibility (NDFD). Five corn varieties were seeded in pots (6 replicates) and grown in a greenhouse. After seeding (5/13/19), replicates were allocated to a water-sufficient treatment (W) or a water-insufficient treatment (D). From seeding to harvesting, W and D pots were watered with 598 and 273 mm of water, respectively. Silking date (date at which 50% of the plants showed silks) occurred on 8/12/19, and harvesting occurred on 9/4/19. At harvesting, 3 internodes and 3 leaf-blades from the bottom of the plant and from the phytomers above the insertion of the ear were dissected and composited by tissue. After drying, tissues were ground to pass through a 2-mm screen (cyclone mill), and 0.25 g was put into Ankom F57 porous bags previously rinsed with acetone to perform in situ digestibility in 3 cows. All tissues of each of the 3 replicates per treatment and variety were incubated within a same cow, so cow was considered a blocking factor. All bags were placed simultaneously into the rumen and incubated for 0, 3, 6, 9, 12, 18, 24, 48, 96, and 240 h. In situ disappearance parameters were determined using Proc NLIN of SAS according to the model $\text{ISNDFD} = B \times [1\text{-}e^{(-k \times T)}]$, where B is the potentially digestible NDF and k is the fractional digestion rate of B. We also measured fraction C at 240 h (i.e., uNDF240). Kinetic parameters were contrasted using a mixed model that included the effects of cow (random), treatment (fixed), hybrid (fixed), treatment × hybrid (fixed), and the residual error. For the lower stems, drought stress did not affect fractions B (65.0%; $P = 0.22$) and C (35.0%; $P = 0.22$) and did not affect k (5.3%/h; $P = 0.34$). For the upper stems, drought stress did not affect fractions B (76.3%; $P = 0.30$) and C (23.8%; $P = 0.30$) but tended to increase k (4.5 vs. 5.2%/h; $P = 0.09$). For the upper blades, drought stress increased fraction B (82.5 vs. 84.3%; $P < 0.02$) and reduced fraction C (17.5 vs. 15.7%; $P = 0.02$) but did not affect k (3.9%/h; $P = 0.42$). In conclusion, under the conditions of this study, drought stress had minimal effects on NDFD.

Key Words: drought, corn silage, fiber digestibility

370 Effect of forage processor roll-gap settings and storage length on the fermentation profile, nitrogen fractions, and kernel processing score of whole-plant corn silage harvested at different maturities. B. A. Saylor*[1], E. C. Diepersloot[1], L. G. Ghizzi[1,2], J. O. Gusmao[1,3], C. Heinzen Jr.[1], C. L. McCary[1], M. R. Pupo[1], H. Sultana[1], and L. F. Ferraretto[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL*, [2]*Department of Animal Nutrition and Animal Production, University of São Paulo, Pirassununga, São Paulo, Brazil*, [3]*Department of Animal Science, Federal University of Lavras, Lavras, Minas Gerais, Brazil.*

The objective of this study was to assess the effect of forage processor roll-gap settings and storage length on the fermentation profile, N fractions, and kernel processing score of whole-plant corn silage harvested at different maturities. Samples from a single corn silage hybrid at 3 harvest maturities [1/4, 1/2, and 3/4 kernel milk line (early, intermediate, and late, respectively)] with 2 roll-gap settings (1 and 3 mm) on a forage harvester were collected at harvest and stored in quadruplicate vacuum pouches for 0, 30, 120, or 240 d. Data were analyzed as a split-split-plot design using the MIXED procedure of SAS. Concentrations of DM tended to differ ($P = 0.09$) among maturities and averaged 30.4, 31.8, and 37.4% for early, intermediate, and late maturity silages, respectively. There was a maturity × storage length interaction for pH ($P = 0.02$) and lactic acid concentrations ($P = 0.05$). Silage pH was similar among maturities at 30 and 120 d, but was greater in late maturity silage at 240 d. Concentrations of lactic acid were similar among maturities at 30 and 120 d, but were greater in intermediate maturity silage compared with late maturity silage at 240 d. Concentrations of acetic, propionic, and total acids were unaffected ($P > 0.05$) by treatments. Concentrations of soluble CP increased ($P < 0.001$) from 20.4 to 44.5% of CP as storage increased from 0 to 240 d. A maturity × storage length interaction was observed ($P = 0.01$) for ammonia-N (% of N) concentrations; with no differences observed at 0, 30, and 120 d, but with greater concentrations in intermediate maturity silage at 240 d. Concentrations of WSC decreased ($P < 0.001$) from 13.3 to 1.9% of DM as storage increased from 0 to 240 d. Kernel processing score increased ($P = 0.01$) from 62.4 to 67.7% of starch passing through a 4.75-mm screen as storage increased from 0 to 240 d. These results reaffirm the effects of prolonged fermentation on silage nitrogen fractions and suggest that prolonged fermentation may increase kernel processing score.

Key Words: corn silage, maturity, roll-gap

371 Fiber digestion kinetics of summer annual grasses with or without brown midrib genotype. G. Ferreira*[1], A. I. Silva-Reis[1,2], A. A. Pereyra[1,3], and C. L. Teets[1], [1]*Department of Dairy Science, Virginia Tech, Blacksburg, VA*, [2]*Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Corrientes, Corrientes, Argentina*, [3]*Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina.*

The objective of this study was to determine fiber digestion kinetics of summer annual grasses containing (BMR) or not (CONV) the brown midrib genotype. Four varieties (2 CONV and 2 BMR) of corn (CN), sorghum (SG), and pearl millet (PM) were planted in 1 m × 3 m plots (3 plots/variety). All plots were planted and harvested on 5/30/19 and 9/18/19, respectively. Samples of leaf-blades, stems, and whole plants were collected, dried, and ground to pass a 2-mm screen, and 0.25 g was put into Ankom F57 porous bags previously rinsed with acetone to perform in situ digestibility in 3 cows. All samples from 1 of the 3 replicates per species and variety were incubated within the same cow, so cow was considered a blocking factor. All bags were placed simultaneously into the rumen and incubated for 0, 3, 6, 9, 12, 18, 24, 48, 96, and 240 h. In situ disappearance parameters were determined using Proc NLIN of SAS according to the model $\text{ISNDFD} = B \times [1\text{-}e^{(-k \times T)}]$, where B is the potentially digestible NDF, and k is the fractional digestion rate of B. Kinetic parameters were contrasted using a mixed model that included the effects of cow (random), species (fixed), genotype (fixed), species × genotype (fixed), and the residual error. For the whole plants, fraction B was 82.1, 73.5, and 70.6% for CN, PM and SG, respectively ($P < 0.01$) and 71.2 and 79.5% for CONV and BMR, respectively ($P < 0.01$). The fractional digestion rate did not differ among species (2.85%/h; $P = 0.85$) but was greater for BMR than for CONV (3.19 vs. 2.51%/h). For the blades, fraction B was 87.2, 82.7, and 82.2% for PM, CN and SG, respectively ($P < 0.01$) and 81.1 and 86.9% for CONV and BMR, respectively ($P < 0.01$). The fractional digestion rate was 3.59, 3.06, and 2.99%/h for PM, SG, and CN, respectively ($P < 0.04$) and 3.56 and 2.87%/h for BMR and CONV, respectively ($P < 0.01$). For the stems, fraction B (62.4%) did not differ among species ($P = 0.12$) and genotypes ($P = 0.24$), and k (2.08%/h) did not differ among species ($P = 0.59$) and genotypes ($P = 0.08$). In conclusion, when comparing species, BMR varieties do not necessarily have greater fiber digestibility than CONV varieties.

Key Words: summer annuals, fiber digestibility, brown midrib (BMR)

372 Relationships between fiber contents and in vitro dry matter digestibility of alfalfa and grass silages. C. Plett*[1], A. Scott[1], K. H. Ominski[1], N. McLean[2], C. Lafreniere[3], S. Bittman[4], and J. C. Plaizier[1], [1]*University of Manitoba, Winnipeg, MB, Canada*, [2]*Dalhousie University, Truro, NS, Canada*, [3]*Université du Québec en Abitibi-Temiscamingue, Rouyn-Noranda, QC, Canada*, [4]*Agriculture and Agri-Food Canada,* *Agassiz, BC, Canada.*

To assess how the fiber contents of silages of perennial forages are related to their in vitro dry matter digestibility (IVTDMD), 270 samples of first cut alfalfa, grass, and alfalfa/grass silages were collected from dairy farms throughout Canada. Samples were analyzed for NDF and ADF by wet chemistry and for IVTDMD using 48 h incubation the ANKOM Daisy II system with an NDF ending. Summary statistics (Table 1) show that the ADF and NDF contents of the samples collected had a wide range in composition and were representative of first-cut alfalfa and alfalfa/grass silages (NRC, 2001. National Research Council, 2001 Nutrient Requirements of Dairy Cows). Pearson correlations between the ADF, NDF, and IVTDMD contents of the samples were determined using the SAS software, as the NDF and ADF contents of forages are routinely used to determine feed intake, energy content and digestibility of ruminant feeds. The correlation coefficients of the regression of ADF on IVTDMD and of NDF on IVTDMD, were −0.41 ($P < 0.01$) and −0.55 ($P < 0.01$), respectively. A reason for the low correlation coefficients may be that a NDF ending, rather than a pepsin ending for the Daisy II method was used, as the former method results in higher dry matter digestibility estimates. Differences in the alfalfa to grass ratio among samples may also have contributed to the low correlations. Our data does not indicate how the IVTDMD data can best be used for quality assessment and diet formulation when AFD and NDF analyses are available. More research may also be needed to modify the Daisy II method to provide more accurate estimate of total-tract dry matter digestibility in cattle.

Table 1 (Abstr. 372).

Item	Min	25th percentile	Median	75th percentile	Max
NDF, % DM	34.1	42.2	46.3	50.9	64.4
ADF, % DM	24.0	30.1	32.8	36.0	49.3
IVTDMD, %	45.3	58.7	63.4	68.5	78.7

Key Words: fiber, in vitro dry matter digestibility, silages

373 The content of lignin and hemicellulose of silages from different genotypes of sorghum biomass.

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In countries with a tropical climate, seasonal productivity of forage is one of the most important factors limiting animal performance under grazing systems, which prevents animals from expressing their maximum genetic potential. Therefore, it is important to search for alternative feedstuff that would meet the demand for roughages during periods of scarcity, such as the sorghum biomass. The objective of this study was to analyze the content of lignin and hemicellulose of silages of different genotypes of sorghum biomass. We evaluated 8 new genotypes of sorghum biomass (B004, B005, B009, B010, B011, B013, B015, and B020), one commercial genotype of sorghum biomass (K1009), and 2 non-biomass commercial genotypes of sorghum that are commonly used for silage (BRS655 and Volumax). The experiment was conducted using a randomized design with different genotypes as treatment and with 4 repetitions of each treatment. The material was ensiled using PVC silos that were kept closed for 45 d. Then, the silos were opened and we measured the content of hemicellulose and lignin. The data were analyzed using one-way ANOVA followed by the Tukey test ($\propto < 0.05$). Statistically significant differences ($P < 0.05$) and great variability were found between the genotypes for both cell wall components. The average hemicellulose content ranged from 23.6% to 28.6% and the average lignin content varied between 4.5% and 6.5%. Among the biomass genotypes, the genotype B020 had one of the lowest contents of hemicellulose (mean ± standard deviation = 25.0 ± 0.44) and lignin (4.5 ± 0.47), with the latter being lower than the commercial biomass genotype ($P < 0.05$). In conclusion, the biomass genotype B020 is a possible candidate for usage in the feeding of ruminant animals because its low hemicellulose and lignin content would not limit its intake and digestibility.

Key Words: alternative feedstuff, feedstuff scarcity, ruminants

374 Effects of pre-cutting round hay bales during baling on forage quality and processing time.

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Innovative round hay balers with knives that cut the hay as it enters the baling chamber reduce the particle size upon baling and eliminating the need for a tub-grinder. The objective of this study was to evaluate the effects of a round hay baler with knives on forage quality at baling and after storage, and the processing time to reduce particle size before feeding. Alfalfa hay was baled (560 M Megawide HC^2, John Deere, Moline, IL) with knives every 15.25 cm (CUT) or without knives (NORM). At baling and after 6 mo storage uncovered, bales were weighed, measured and 10 core samples obtained for nutrient analysis. Cores were separated into outer 15 cm and inner 15 to 46-cm segments to determine depth of spoilage. After storage, particle size was reduced to approximately 4 inches using a mixer wagon for CUT or a tub grinder for NORM. Grinding time and hay loss were analyzed as a completely randomized design. Bale dimensions, weight and density were analyzed as a split-plot design, while nutrient analysis was analyzed as a split-split plot design with treatment as the whole plot, storage period as the sub-plot, and core depth as the sub-sub-plot. Compared with NORM, CUT increased bale weight (513 vs. 581 ± 19.8 kg DM; $P < 0.001$) and density (154 vs. 170 ± 5.9 kg DM/m^3; $P < 0.001$). Core depth interacted with storage time point whereby ADF concentration increased more for outer than inner cores from baling to storage (2.6 vs. 1.1 ± 0.48%; $P < 0.01$), with similar effects for lignin (0.7 vs. 0.2 ± 0.11%; $P < 0.001$) and 240-h uNDF (1.9 vs. 0.8 ± 0.52%; $P = 0.01$). Compared with NORM, CUT increased concentrations of ADF and aNDFom by 0.6% ($P = 0.01$), and decreased RFQ (117 vs 112 ± 3.2 points; $P = 0.02$). The CUT treatment increased time to reduce particle size (11.0 vs 3.6 ± 1.53 min; $P < 0.001$), but decreased grinding DM loss by 24.1 ± 2.83 kg ($P < 0.001$). In summary, CUT produced larger, more dense bales and increased fiber content slightly; however, the increase may be negligible in ration formulation when hay is fed as part of a TMR. In view of DM loss advantages for CUT during grinding, post-grinding nutrient analysis should be considered.

Key Words: processing, harvest, shrink

Physiology and Endocrinology

375 Blood microRNA profile differs between primiparous and multiparous cows fed a high-concentrate diet. N. Reisinger*[1], A. Stauder[2], M. Hackl[3], J. Faas[1], and Q. Zebeli[2], [1]*BIOMIN Research Center*, *Tulln, Austria*, [2]*Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, Vetmeduni Vienna*, *Vienna, Austria*, [3]*TAmiRNA GmbH*, *Vienna, Austria*.

During transition period dairy cows have to deal with several stressful events e.g., change from a fiber-rich close-up diet to a high-concentrate diet. Primiparous cows (PPC) might be more susceptible to these changes than multiparous cows (MPC). The aim of the study was to investigate the blood microRNA (miRNA) profile of PPC and MPC before and during feeding of a high concentrate diet. Eight lactating Simmental cows (73 ± 25.4) DIM; 4 PPC, 4 MPC) were used for this study. Cows received a baseline diet (60% roughage; 40% concentrate) followed by a high concentrate diet (HCD) for 4 weeks (40% roughage, 60% concentrate). Blood was collected during baseline diet, wk 1 and wk 4 of HCD. miRNAs were isolated from plasma samples and sequenced using the CleanTag small RNA library prep kit. Sequencing was performed on an Illumina NextSeq 550. Reads were mapped against miRBase using miRDeep2. MiRNA raw read counts were processed using EdgeR and generalized linear models to perform differential expression analysis. P-values were adjusted for multiple testing using Benjamini-Hochberg false discovery rate. Overall, 48 miRNAs were differently expressed in PPC and MPC ($P < 0.05$). During baseline, 16 miRNAs were differently expressed in PPC cows compared with MPC ($P < 0.05$). While during wk 1 and 4 of HCD, 26 and 10 miRNAs were differently expressed ($P < 0.05$), respectively. Differential expression was confirmed for a subset of miRNAs by RT-qPCR. Accordingly, the HCD increased the expression of bta-miRNA-122 by 2.2-fold as well as the expression of bta-miR-30a-5p by 1.6-fold in wk 4 ($P < 0.05$). These 2 miRNAs were as well affected by parity in wk 1 of the HCD as they were increased in PPC by 3.6-fold and 2.9-fold ($P < 0.05$), respectively. In addition, bta-miR-192 was increased by 1.5-fold, bta-miR-194 by 1.4-fold and bta-miRNA-100 by 2.0-fold in PPC during wk 1 of the HCD ($P < 0.05$). Furthermore, bta-miRNA-1246 was increased by 2.1-fold in PPC ($P < 0.05$). In conclusion, HCD affected miRNAs associated with liver and mammary gland health in both PPC and MPC cows. However, other miRNAs associated with the gut and immune system were differently affected in PPC and MPC. Therefore, PPC might need special attention and might benefit from different management.

Key Words: blood miRNA, parity, high-concentrate diet

376 Fatty acid-induced ORAI1 facilitates endoplasmic reticulum stress through mitochondrial dysfunction in calf hepatocytes. B. Zhang[1], M. Li[2], W. Yang[2], C. Xia[2], H. Zhang[2], and C. Xu*[2], [1]*College of Life Science and Technology, Heilongjiang Bayi Agricultural University*, *Daqing, Heilongjiang, China*, [2]*College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University*, *Daqing, Heilongjiang, China*.

High-producing dairy cows experience negative energy balance (NEB) during early lactation, which is associated with mobilization of nonesterified fatty acids (NEFA) from adipose depots. In non-ruminants, store-operated Ca^{2+} entry moiety ORAI1 regulates hepatic lipogenesis by mediating Ca^{2+} homeostasis. The present study aimed to determine whether fatty acids activate oxidative stress via altering intracellular Ca^{2+} signaling and ORAI1, thereby inducing mitochondrial dysfunction and endoplasmic reticulum (ER) stress. Calf hepatocytes from 4 healthy female calves (1 d old, 40–50 kg) were used to perform an in vitro challenge with a 1.2 m*M* mixture of fatty acids. including *cis*-9–18:1, *cis*-9,*cis*-12–18:2, 16:0, 18:0, and *cis*-9–16:1 for 0, 0.5, 1, 3, 6, 9 and 12 h. Furthermore, transfecting calf hepatocytes with small interfering ORAI1(siORAI1) or the ORAI1 inhibitor BTP2 for 24 h followed by a challenge with the 1.2 m*M* mixture of fatty acids for 6 h and sarcoendoplasmic Ca^{2+} ATPase inhibitor thapsigargin or the calcium ionophore ionomycin for 6 h, respectively, to determine ORAI regulation. Data were submitted for ANOVA testing using a mixed model for repeated measurements. There was a decrease ($P < 0.05$) in intracellular reduced glutathione (GSH) and superoxide dismutase (SOD) by 0.5 h, significantly elevated MDA and hydrogen peroxide by 1 h ($P < 0.01$), and significantly increase ($P < 0.01$) in the major ER stress proteins PERK, IRE, ATF6, and GRP78 by 6 h. Exogenous fatty acids upregulated ORAI1 mRNA and protein abundance, and VDAC1, CLPP, and CypD protein abundance ($P < 0.01$). In addition, flow cytometry showed that fatty acids increased mitochondrial membrane potential ($P < 0.05$) and induced transient opening of mitochondrial permeability transition pore (mPTP). The reactive oxygen species (ROS) inhibitor NAC blocked ORAI1 and major ER stress proteins. Furthermore, compared with controls, BTP2 or siORAI1 abrogated oxidative stress including increased GSH and SOD content, decreased MDA and hydrogen peroxide content ($P < 0.01$), and decreased ROS production. ER stress protein abundance also was downregulated and mitochondrial function was restored. Furthermore, increased ORAI1 expression by thapsigargin, or ionomycin induced mitochondrial dysfunction. Overall, these data suggest that ORAI1 mediates fatty acid-induced ER stress through controlling mitochondrial dysfunction and oxidative stress.

Key Words: ORAI1, mitochondria dysfunction, endoplasmic reticulum

377 Healthy mammary quarters neighboring LPS-infused quarters undergo hypogalactia associated with transcriptional changes in immune and metabolic genes. E. M. Shangraw*[1], R. O. Rodrigues[1], R. K. Choudhary[2], F.-Q. Zhao[2], and T. B. McFadden[1], [1]*University of Missouri*, *Columbia, MO*, [2]*University of Vermont*, *Burlington, VT*.

Infusion of lipopolysaccharides (LPS) into a mammary gland can provoke symptoms in healthy neighboring glands, termed the systemic effect. Our aim was to determine how localized mastitis affects the mammary transcriptome of neighboring glands in lactating dairy cows, focusing on the early progression of the inflammatory response. Ten multiparous cows were paired based on milk yield, parity, and SCC. Within pair, cow treatments were randomly assigned: 1) LPS treatment (T) or 2) control (C). Within cow, udder-halves were randomly assigned to sub-treatments: in T cows, ipsilateral front and rear quarters received an infusion of 50µg LPS in 10mL saline (TL); contralateral quarters received 10mL saline (TS). In C cows, udder-halves received either 10mL saline (CS) or no infusion (CN). Rear quarters were biopsied at 0, 3 and 12h post-infusions. Total RNA isolated from TL, TS, and CS mammary tissue at 3 and 12h was sequenced to a depth of 44.1 ± 2.4 million reads per sample. Transcripts were declared significant at FDR $P < 0.05$, log2 fold change $> |1.0|$, and were annotated to KEGG pathways. Comparing mammary transcriptomes of T and C cows revealed 2,779 and 1,363 differentially expressed (DE) genes at 3 and 12h, respectively. Of these genes, > 95% were DE only in TL and represented classic LPS immune responses at both times. Interestingly, despite the relatively weak response, TS quarters underwent hypogalactia to a similar extent as TL, as 12h milk yields of non-biopsied front quarters declined to 47 ± 5% and 52 ± 9% of 0h for TS and TL, respectively. To determine if a systemic mediator was the cause, we focused on genes DE in both TL and TS. At 3h, TNF, NF-κβ and NOD-like receptor signaling pathways were identified. At 12h, PPAR signaling and arachidonic acid metabolism were identified. Two genes, FKBP5 and LYVE1, were upregulated in TS and TL at both times. In summary, neighboring glands are initially affected by a transient systemic immune response triggered in the infused gland. Later responses shifted to changes in cellular metabolism, perhaps explaining the decline in milk yield.

Key Words: mastitis, mammary, transcriptome

378 Changes in biomarkers of metabolic stress during late gestation of dairy cows associated with colostrum volume.

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The objective of this observational study was to compare the metabolic status of dairy cows during the last 6 wk of gestation based on colostrum volume. Healthy Holstein cows were randomly selected from 2 commercial dairy herds in Michigan. Two cohorts of 21 cows each, one for the summer and another for the fall of 2019, were enrolled in each farm with similar parity distribution. Cows were blood sampled weekly during the last 6 wk of gestation, and biomarkers related to nutrient utilization, oxidant status, and inflammation were quantified in serum. Cows were milked within 6h of calving and the volume of colostrum produced was recorded and an aliquot collected. For this study, only the data from 63 cows producing colostrum with IgG >50 g/L were considered to avoid confounding due to poor IgG content. Cows were grouped into high colostrum producer (HCP) or low colostrum producer (LCP). For that, we arbitrarily defined 6 L of colostrum (4 L for first and 2 L for second feeding of calves), as the cut-off point. Data were analyzed statistically through mixed models with repeated measures including the fixed effects of group (HCP vs. LCP), time, and their interaction; and the random effects of cow, lactation number, and farm. The HCP cows had higher β-hydroxybutyrate, blood urea nitrogen, magnesium, and lower glucose serum concentrations throughout the study period compared with LCP cows. Furthermore, HCP cows also showed higher concentration of reactive oxygen species compared with LCP cows, resulting in higher oxidant status index values despite no changes in antioxidant potential. No differences were found for haptoglobin, a biomarker of inflammation. Collectively, these data show that HCP cows had greater utilization of nutrients throughout the study period. Thus, indicating a higher metabolism activity, which might explain the elevation in markers of oxidant status. Nevertheless, the differences observed did not result in changes in biomarkers of inflammation or lipid mobilization, suggesting that physiological homeostasis was not disrupted in HCP cows during late gestation.

Key Words: oxidant status, colostrogenesis, passive immunity

379 Glucose tolerance appears to follow a daily rhythm in dairy cows.

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Glucose is an important metabolic fuel for dairy cows that is required for milk lactose and fat synthesis, and other essential processes. Glucose metabolism is regulated by circadian rhythms, repeating cycles of ~24 h that govern many aspects of behavior and physiology. It is well-established that blood glucose and insulin concentrations follow 24 h rhythms in dairy cows, but it is unknown if glucose tolerance and insulin sensitivity follow a 24 h rhythm. To determine if glucose tolerance and insulin sensitivity follow a 24 h rhythm in dairy cows, 12 multiparous lactating Holstein cows were enrolled in a randomized block design (2 blocks; n = 6 per block). Within each block, cows were subjected to intravenous glucose tolerance tests (IVGTT) at 0300, 0900, 1500 and 2100. During IVGTT, cows were fed 12x/d at 2 h intervals to stabilize feed intake across the day. For each IVGTT, 250 g of a 50% wt/vol D-glucose solution were infused via jugular catheter and blood was collected at −15, −5, immediately before, 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, and 240 min relative to infusion. Insulin and glucose kinetics were modeled using WinSAAM ver. 3.0.8. Glucose concentration fit a 2-compartment model with a delay component for glucose recycling. Effect of time of day on baseline glucose and insulin concentrations, glucose half-life, and insulin resistance - measured by post-IVGTT increase in insulin concentration- were analyzed in SAS 9.4 using a mixed model including main effects of time of day and block, and the random effect of cow. Moreover, time course data were fit to the linear form of cosine functions using SAS 9.4 and analyzed for fit of a 24 h rhythm using a zero-amplitude test. Half-life of glucose was affected by time ($P = 0.05$), with half-life being 25% greater at 0300 than 0900 and 1500 ($P < 0.05$), and half-life being 25% greater at 2100 than 1500 ($P < 0.05$). Moreover, half-life of glucose fit a cosine function with a period of 24 h, indicating a daily rhythm ($P = 0.005$). Baseline insulin concentration differed across the day with insulin concentration being greatest at 0300, and lowest at 0900 ($P = 0.0002$), while baseline glucose concentration was not affected by time of day ($P = 0.30$). Insulin sensitivity differed across the day, being greater at 0300 and 1500 than 0900 and 2100 ($P < 0.05$). Results suggest that glucose tolerance follows a daily rhythm in dairy cows, with greater glucose tolerance in morning and afternoon compared with overnight.

Key Words: daily rhythm, glucose metabolism, circadian

380 Glucose infusion during heat stress restores normoglycemia but does not improve milk production.

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Mild hypoglycemia is one of a myriad of physiological changes typically experienced by lactating dairy cows exposed to heat stress (HS); the consequences of which are not yet well defined or understood. The objective of this experiment was to infuse dextrose to re-establish normoglycemia during HS, hypothesizing that bringing the cow out of hypoglycemia would improve milk production during HS. Multiparous lactating Holstein cows (n = 6; 63.33 ± 2.35 DIM, 3.17 ± 0.40 lactations, 316.38 ± 13.93 kg of BW) were housed in temperature-controlled rooms and subjected to a thermoneutral period (TN; 10 d; 18 ± 4°C) followed by a HS period (15 d; 33 ± 4°C). Cows were offered a total mixed ration and water for ad libitum consumption, and feed refusals were recorded once daily. All cows were milked 2 times per day and milk yields were recorded after each milking. Rectal temperatures and respiration rates were measured once daily, and both indices increased during HS (38.18 ± 0.07 vs 39.44 ± 0.09°C, respectively; $P < 0.01$ and 28.86 ± 1.2 vs 73.80 ± 3.00 breaths per min, respectively; $P < 0.01$). Circulating glucose concentrations were determined during TN and HS conditions with hand-held glucometers using blood collected from indwelling jugular catheters. As expected, blood glucose concentrations declined with HS (61.63 ± 1.58 and 55.85 ± 1.73 mg/dL for TN and HS, respectively; $P < 0.04$). Over the last 96 h of the HS period, dextrose was continuously infused at rate that returned each individual cow's circulating glucose concentrations to those observed during TN (100 ± 10% of TN values). During TN conditions, both milk production (50.44 ± 1.21 kg) and feed intake (49.02 ± 1.44) were at their highest ($P < 0.01$). Milk production decreased under HS conditions (36.49 ± 1.23 kg) but did not improve with dextrose infusion (33.99 ± 2.02 kg; $P = 0.76$). Feed intake also decreased under HS conditions (35.58 ± 2.25 kg,) and did not improve with dextrose infusion (27.27 ± 3.46 kg; $P = 0.17$). The results of this experiment indicate that, although cows become hypoglycemic during heat stress, this reduction in circulating glucose concentrations is not limiting milk production.

Key Words: heat stress, glucose, milk production

Ruminant Nutrition: Gut Physiology, Fermentation, and Digestion

381 Factors influencing fiber digestibility in dairy cows. D. Cavallini*[1,2], A. Concolino[1], L. Mammi[1], G. Canestrari[1], S. Speroni[1], G. Buonaiuto[1], L. Campidonico[1], F. Ghiaccio[1], A. Palmonari[1], E. Valle[2], and A. Formigoni[1], [1]*Department of Veterinary Sciences, University of Bologna*, *Ozzano Emilia, Italy*, [2]*Department of Veterinary Sciences, University of Turin*, *Grugliasco, Italy*.

Fiber digestibility (FD) is a main topic on dairy cows rationing. Main effects on FD could be attributed to pH as well as other aspects. The objective of this study was to investigate in high producing cows, the factors that could have a major impact on total-tract fiber digestibility (TTD). A total of 7 studies involving 53 dairy cows and 1364 fecal samples were examined. Performances and rumen parameters of those cows were recorded10d before the fecal sampling. All data were statistically analyzed in JMP (v14.3). K-means cluster analysis was used to detect cows within diet and trial based on higher or lower TTD (HD and LD group respectively). Then cows with variation in TTD due to diet (group V, n = X) were divided from cows with HD or LD constant and independent from the diet (group I, n = X). Mixed model procedure with a factorial arrangement for TTD, with day as repeated measure was used to study HD and LD groups by V or I. LD and HD -V, did not differed in any parameter analyzed, except for milk yield (+1.3kg in HD, $P = 0.04$). In fact, these differences were related to TMR characteristics (+2.5% of $uNDF_{240h}$, $P < 0.01$) in diets fed LD-V. I groups differed in BW (+95kg in HD, $P < 0.01$) and lactation number (1.5 vs. 3 in LD and HD respectively, $P < 0.01$). Rumen parameters were characterized by lower rumination and pH on HD-I (−72 min/d and −0.34 pH point/d respectively, $P < 0.01$). These results showed that diet composition could affect FD, and that differences in TTD within cow led to increasing production and efficiency. On the other hands, when the differences in TTD are linked to the individual cow the main discriminant effect is the cow size and the lactation number, with reflections on rumen parameters such as rumination and pH.

Key Words: fiber digestibility, dairy cows, pH

382 Development of an in vitro assay to determine the intestinal digestion of lipids in ruminants. J. R. Vinyard*[1], E. Sarmikasoglou[1], S. L. Bennett[1], J. Arce-Cordero[1], G. Aines[2], K. Estes[2], C. Zimmerman[2], and A. P. Faciola[1], [1]*Department of Animal Sciences, University of Florida*, *Gainesville, FL*, [2]*Balchem Corporation*, *New Hampton, NY*.

The objective of this study was to develop an in vitro assay to determine the extent of intestinal digestion of corn oil (CO), canola oil (CA), and beef tallow (BT) via manipulation of incubation length and concentrations of lipase, bile, and calcium within a buffer solution. The buffer solution (0.5 M KH_2PO_4) was raised to a pH of 7.6 using 5% NaOH and made to volume using distilled water. Unless otherwise stated, 0.5 g of each lipid source were incubated separately and in triplicate, with triplicate runs for each treatment in 40 mL of KH_2PO_4 for 24 h with pancreatin (ENZ), bovine bile, and $CaCl_2$ included at 8 g/L, 2.5 g/L, and 10 mM, respectively. Individually, concentrations of ENZ, bile, and $CaCl_2$, as well as incubation length were tested (Table 1). Free glycerol (GLY) and fatty acid (FFA) concentrations were measured using colorimetric assays as determinants of digestibility. Data were analyzed as a completely randomized block design, with run used as block, using the Glimmix procedure of SAS. For each lipid source, GLY increased with increasing ENZ; however, FFA was lowest at 0 g/L ENZ but was similar at 6, 8, and 10 g/L. Both GLY and FFA were greater for 2.5 and 5 g/L of bile than for 0 g/L for each lipid source. Calcium concentration did not affect GLY or FFA for either CO or CA; however, GLY and FFA for BT were greater when calcium was included at 5 and 10 mM than at 0 mM. For all fat sources, GLY and FFA increased after 1 h until 12 h, but did not increase from 12 to 24 h. These results indicate that GLY and FFA can be used as determinants of lipid digestibility when lipid source is incubated for at least 12 h in a buffer solution containing 8 g/L ENZ, 2.5 g/L bile, and 5 mM.

Table 1 (Abstr. 382). Levels of each variable used to develop a representative assay for determining in vitro lipid intestinal digestibility

Variable	Range
Pancreatin	0, 6, 8, and 10 g/L
Bile	0, 2.5, and 5 g/L
Calcium	0, 5, and 10 m*M*
Incubation length	1, 3, 6, 9, 12, and 24 h

Key Words: digestibility, free fatty acid, glycerol

383 Alterations in ruminal and fecal microbial communities of dairy cows during ketosis. Q. Wang*[1], W. Bao[1], Y. Cui[1], J. J. Loor[2], B. Gao[1], Y. Ren[1], and C. Xu[1], [1]*College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University*, *Daqing, Heilongjiang, China*, [2]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois*, *Urbana, IL*.

The objective of this study was to simultaneously investigate the profiles of rumen and fecal microbiome in cows afflicted by clinical ketosis. Twenty-seven Holstein dairy cows of similar age, parity and body condition score were used in the study. Blood hydroxybutrate concentration was 4.1 ± 0.72 mmol/L in ketotic cows, 1.36 ± 0.12 mmol/L in subclinical ketotic cows, and 0.88 ± 0.14 mmol/L in healthy control cows. Rumen fluid and fecal samples were all collected at 21 d postpartum in the morning within 2 h before feeding. An oral tube with a syringe was used for rumen sampling, while fecal samples were collected via rectal grab. Bacterial communities were identified by 16S rRNA sequencing. We found that the microbial structure, composition and diversity were different in rumen and feces and affected by ketosis compared with healthy dairy cows. More OTU encompassing bacterial genus and species were detected in rumen fluid than feces among dairy cows in the 3 groups. Compared with healthy cows, both species richness and evenness were higher in rumen fluid from clinical and subclinical ketotic cows. Microbial composition in rumen fluid and feces differed between ketotic and healthy cows. Regardless of ketotic status, principal component analysis (PCA) revealed that there were more differences at the phyla and genus level between rumen fluid and feces. Regardless of health status, microbial composition distribution at the phylum level revealed that *Firmicutes, Prevotella, Bacteroidetes, Clostridiales, Lachnospiraceae* were the top 5 dominant species in rumen fluid and fecal samples. A total of 38 bacteria were detected as dominant species in which *Prevotella* and *Butyrivibrio* were the top 2 commensal bacteria in rumen fluid.Overall, the Clinical-ketosis group was significantly enriched for the genera *Rumenococcaceae*, *BS11, RF16, Christensenellaceae, Anaeroplasma* and *Mogibacteriaceae* when compared with the healthy group. Hence, ketosis increased the diversity of bacteria with important roles in metabolism of amino acids and energy substrates in rumen fluid.

Key Words: ketosis, rumen fluid, bacterial structure

384 A new pathway for forming acetate during fermentation in bacteria. B. Zhang*[1], C. Bowman[1], and T. Hackmann[1], [1]*University of California-Davis*, *Davis, CA*, [2]*University of Florida*, *Gainesville, FL*.

Acetate is an important VFA formed during fermentation in the rumen. Biochemical pathways for forming it during fermentation in bacteria have been studied for over 80 years. Here, we found a biochemical pathway for forming acetate that was unknown in bacteria. We performed experiments with the skin bacterium *Cutibacterium granulosum* because its genome does not encode any known pathways. With enzymatic assays, we found it forms acetate and uses a pathway involving 2 enzymes. The first enzyme, succinyl-CoA:acetate CoA-transferase (SCACT), forms acetate from acetyl-CoA. The second enzyme, succinyl-CoA synthetase (SCS), synthesizes ATP. The SCACT/SCS pathway is common in eukaryotes, but it has not been found in bacteria. When we searched genomes for bacteria known to form acetate, we found nearly 1/5 encoded the pathway. The next step is to investigate if this pathway is used by rumen bacteria. The search of genome sequences showed that several rumen bacteria encode the pathway. This includes propionibacteria that are close relatives to the bacterium studied here. The impact of the work is that it can guide genetic engineering or other attempts to manipulate acetate production in the rumen.

Key Words: fermentation, rumen, bacteria

385 Gas production kinetics of in situ washout and remaining fractions.

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Objective was to determine if material washed out and remaining in Ankom Dacron bags represents that of the intact sample. Nine corn grains with DM (52.2–82.5%), starch (60.9–74.0%DM), and soluble protein (16.7–54.1%CP) were used. Washout of DM was determined using samples ground to pass a 4mm screen that were agitated for 1min in 30 mL distilled water. Bags were dried at 55°C overnight. Coefficient of variation of triplicate DM remaining in bags was $< 3\%$. Washout ranged from 4.69 to 52.6%DM. Gas production (GP) kinetics were determined by placing Dacron bags (0.5g, 4-mm grind) in 30 mL of Goering & Van Soest (1970) medium and agitating for 1min. Bags were removed, cut open and placed in a second bottle with 30 mL of medium. An intact sample (0.5g) was also fermented in 30 mL of medium, which provided 3 treatments: washout (W), remaining (R), and intact (I). Inocula were blended ruminal fluid from 2 Jersey steers (Diet = 54.5%DM, 13.1%CP, 30.7%aNDFom, 31.3%starch as DM). In vitro GP was measured for 48h in 3 runs using Ankom RF modules. Differences in GP kinetics per g of W, R and I (calculated from DM washout) were detected by aov() in R using the model: $Y = \mu + \text{Run} + \text{Treatment} + \text{Error}$. Single rates of gas production were different for W vs. I (11.0 vs. 13.3%/h, respectively), but R (12.4%/h) was similar to both W and I ($P = 0.007$). Lag time was different for W, R, and I (0.65 vs. 4.88 vs. 4.24h, respectively ($P < 0.0001$). Maximum GP was also different for W vs. I, but R was not different from W or I ($P < 0.0001$). Washout had shorter lags and greater gas production than I, but rates were slower than published values for soluble sugars. Gas production during early fermentation (1 to 9h) was highest for W while R and I were similar ($P < 0.0001$). Gas production was different for all 3 treatments at 12h ($P < 0.0001$) and this difference continued through 24h ($P < 0.0001$). After that, W produced more gas per g DM than R and I ($P < 0.0001$), while R and I were similar ($P > 0.05$). Results indicate that washout and that remaining in bags are distinct fractions that may not represent the intact sample. Similar results were observed when GP was expressed per g of intact sample.

Key Words: fermentation, starch

386 Stimulation of microbial protein synthesis by branched-chain volatile fatty acids (BCVFA) in dual-flow cultures varying in forage and PUFA concentrations.

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Branched-chain amino acids (BCAA) are deaminated by amylolytics to BCVFA, which are growth factors for cellulolytic bacteria that cannot degrade or synthesize their own BCAA. Our objective was to assess dietary conditions that increase the uptake of BCVFA. BCVFA should increase stimulation of cellulolytic bacteria in high forage (HF) diets. However, in low forage (LF) diets, amylolytic bacteria should outcompete for BCVFA. Supplemental PUFA should inhibit cellulolytic bacteria growth, but additional BCVFA should minimize that inhibition. Supplementation of BCVFA could therefore increase efficiency of microbial protein synthesis in LF, HF, and high PUFA diets. The study was an incomplete block design with 8 dual-flow continuous cultures used in 4 periods with treatments ($n = 4$) arranged as a 2×2×2 factorial. The factors were: HF or LF (67 or 33% forage), without or with supplemental corn oil (CO, 3% DM), and without or with 2.15 mmol/d each of isovalerate, isobutyrate, and 2-methylbutyrate (MB). Forage was 33:67 alfalfa:orchard grass pellets, concentrate was mostly ground corn and soybean meal, and diets were isonitrogenous. Data were analyzed with PROC MIXED in SAS (v. 9.4, SAS Institute 2015) with random effects of period and fermenter and fixed effects of diet, CO, BCVFA, and their interactions assessed using ANOVA. The main effect of supplementing BCVFA increased ($P = 0.10$) NDF digestibility (NDFd) by 2.97% units, and CO increased ($P = 0.07$, Diet × CO) NDFd by 6.46% units in LF diets. BCVFA and HF increased ($P < 0.03$) bacterial N by 1.54 and 1.84 g/kg OM truly degraded, respectively. Total BCVFA net production (total outflow × concentration corrected for dose) was lower ($P < 0.01$) with HF compared with LF. When CO and BCVFA were supplemented with LF, total BCVFA net production decreased ($P = 0.10$, Diet × CO × BCVFA). When BCVFA was supplemented with CO, MB net production decreased ($P = 0.08$, CO × BCVFA) with HF, but even more with LF. Providing supplemental BCVFA improved efficiency of cellular growth of cellulolytics and therefore NDFd, which should improve feed efficiency in dairy cows.

Key Words: branched-chain volatile fatty acids (BCVFA), cellulolytic, PUFA

Animal Health: Mastitis 2

387 Effect of treatment at dry-off with intramammary antibiotics, internal teat sealants, or both on the health of dairy cows. W. R. ElAshmawy*[1,2], E. Okello[1,3], D. R. Williams[1], R. J. Anderson[4], P. Rossitto[1], J. D. Champagne[1], K. Tonooka[1], K. Glenn[1], B. Karle[5], T. W. Lehenbauer[1,3], and S. S. Aly[1,3], [1]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California-Davis, Tulare, CA*, [2]*Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University*, *Giza, Egypt*, [3]*Department of Population Health & Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA*, [4]*Animal Health Branch, California Department of Food Agriculture*, *Sacramento, CA*, [5]*Cooperative Extension, Division of Agriculture and Natural Resources, University of California*, *Orland, CA*.

Prevention and control of mastitis continues to be one of the major challenges facing the dairy industry. Intramammary antibiotic (AB) infusion at dry-off is used to treat subclinical mastitis at dry-off and prevent clinical mastitis during the dry period. In addition, internal teat sealants (ITS) are used to prevent exposure to new infections during the dry period. A block randomized trial was completed between December 2016 and August 2018 on 8 California dairy herds. A total of 1,273 cows were block randomized to 1 of 4 treatments (None, AB, ITS, or both) over 2 seasons, summer and winter. Composite milk samples were collected at enrollment (dry-off) and within 14 d after calving for bacteriological culture. The objectives of this clinical trial were to compare the health outcomes between the treatment groups, specifically: 1) clinical mastitis and culling during the dry period and the first 150 d in milk (DIM) in the subsequent lactation, and 2) bacteriological cure and new infections in the first 150 DIM in the subsequent lactation. Logistic regression models showed no significant differences in the odds of clinical mastitis or culling between cows treated at dry-off with AB, ITS, or both compared with the controls (None). Cows treated with both AB and ITS had the highest odds of bacteriological cure (OR 3.05; $P < 0.01$), followed by cows treated with AB (OR 2.38, $P < 0.01$), followed by ITS (OR 2.0; $P < 0.01$) in comparison to controls. Cows treated with AB and ITS at dry off had the lowest odds (OR = 0.45; $P < 0.01$) of developing new infections after calving followed by cows that received internal teat sealants (OR = 0.51; $P < 0.01$); however, cows that received only AB had numerically lower odds (OR = 0.70; $P = 0.09$) in comparison to untreated cows. The most common bacteria isolated by culture at dry off, post-calving and at the first mastitis were Coagulase negative staphylococci, *Streptococcus* spp., *Corynebacterium* spp., *Aerococcus* spp., *Coliforms* and *Lactococcus* spp. Dry cow treatment with AB and/or ITS increased the bacteriological cure and reduce the new infections.

Key Words: dry cow therapy, mastitis, clinical trial

388 Impact of subclinical mastitis detected in the first month of lactation on milk yield, fertility, and culling of dairy cows on USDA-certified organic herds. L. Fernandes*[1], I. Guimaraes[1], N. Noyes[2], L. Caixeta[2], and V. Machado[1], [1]*Texas Tech University*, *Lubbock, TX*, [2]*University of Minnesota*, *St. Paul, MN*.

It is well established that subclinical mastitis (SCM), characterized by somatic cell count (SCC) > 200,000 cell/mL, has a negative impact on productivity, reproductive performance, and survivability of cows from conventional dairy herds. However, information about the detrimental impacts of SCM in dairy cows from organic herds is scarce. Therefore, our objective was to evaluate how SCM diagnosed during the first month of lactation impacts milk production, fertility and culling of cows on organic farms. Data from 2 USDA-certified organic dairy herds located in Texas and New Mexico were extracted from the farms' database software. A total of 2,716 cows that calved between June 2018 and May 2019 were included in the study. Cows with SCC >200,000 cells/mL in the first month of lactation were considered as having SCM. Statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Repeated measured ANOVA models were used to assess the effect of SCM on monthly milk production and SCC linear scores. Cox proportional hazards models were used to evaluate the effect of SCM on the risk of pregnancy and culling. The variables lactation group (1, 2, and 3 or greater), herd, twin parturition, and stillbirth were offered to all models. The prevalence of SCM in the first month of lactation was 32.8%. Subclinical mastitis decreased the milk yield during the first 10 mo of lactation. The average milk yield for cows with SCM and healthy counterparts was 33.1 kg/d and 31.8 kg/d, respectively ($P < 0.01$). Cows with SCM in the first month of lactation had elevated SCC throughout the entire lactation. The average SCC linear score was 4.2 and 2.6 for SCM and healthy cows. Risk of pregnancy was not impacted by SCM ($P = 0.44$). Cows that had SCM in the first month of lactation were 1.82 times more likely to be culled or die than healthy cows ($P < 0.01$). In conclusion, SCM in the first month of lactation impairs milk production, increases the risk of culling, but does not impact fertility of dairy cows under organic certified management.

Key Words: organic, subclinical mastitis, milk yield

389 Pattern of behavioral, physiological, and performance parameters before and after clinical diagnosis of mastitis. M. M. Pérez*[1], E. M. Cabrera[1], C. Rial[1], D. V. Nydam[2], and J. O. Giordano[1], [1]*Department of Animal Science, Cornell University*, *Ithaca, NY*, [2]*Department of Population Medicine and Diagnostic Sciences, Cornell University*, *Ithaca, NY*.

The objective of this study was to compare the pattern of multiple behavioral, physiological, and performance parameters automatically collected by sensors in cows diagnosed with clinical mastitis (CM). Data from Holstein cows (n = 1,206) evaluated for CM by clinical examination at or after milking for up to 28 DIM were used. Sensor parameters used were: activity (ACT), resting (RET), rumination (RUM) and eating (EAT) behavior, reticulo-rumen temperature (TEMP), milk production (MILK) and milk components (FAT, PROT, LACT), and milk conductivity (CON). Sensor data from d −7 to 7 after CM diagnosis was compared between cows with no evident signs of clinical disease (NCD n = 824), CM only (CMO; n = 52), and CM plus another disorder during the 15-d period (CM+ n = 29). Data were analyzed by ANOVA with repeated measurements with group, time, and their interaction and lactation number (1, 2, or 3+) as fixed effects. Cow within group was a random effect and the subject of repeated measures. Sensor data used for NCD cows was for the 7 d before and after the average DIM at mastitis diagnosis for cows in the CMO and CM+ group. MILK was reduced ($P < 0.01$) after d −5 for CM+ and after d −2 for CMO compared with NCD, with the greatest difference on d 1 and 2. FAT and FAT-to-PROT ratio values differed ($P < 0.01$) in the range of d −1 to 2 as they were greater for CMO and CM+ than for NCD. CON was greater ($P < 0.01$) for CMO and CM+ than for NCD after d −3 and d −2, respectively with the greatest difference in d −1 and 2. RET was greater ($P < 0.01$) for CMO than NCD after d 0. ACT was lesser ($P < 0.01$) for CM+ than NCD after d 0. RUM was lesser ($P < 0.01$) for CMO and CM+ from d −5 to d 5 with the greatest difference on d 0, whereas EAT was lesser ($P < 0.01$) for CMO and CM+ from d −2 to 7 d with the greatest difference on d 0 and 1. TEMP was greater ($P < 0.01$) for CM+ than NCD on d −1 and 0. We conclude that cows with CM only and CM plus another disorder presented specific patterns of behavioral, physiological, and performance parameters that can be detected by sensors hence, these pattern changes might be used to predict the occurrence of cases of CM.

Key Words: sensor, mastitis, dairy cow

390 Impact of dry-off management in robotic milking systems on milking behavior, milk yield, and somatic cell count. A. E. France*[1], S. Dufour[2], D. F. Kelton[3], H. W. Barkema[4], D. Kurban[2], and T. J. DeVries[1], [1]*Department of Animal Biosciences*, *Guelph, ON, Canada*, [2]*Faculté de*

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The objective of this study was to determine the effect of dry-off management of cows milked in automatic milking systems (AMS) on milk yield, milking behavior, and SCC. In 5 commercial dairy farms in Quebec, Canada, 341 cows were assigned to 1 of 4 treatments for 2-wk before dry off: 1) reduced milking (RM: 2x/d or if expected to yield 17 kg/milking; n = 95), 2) reduced feeding (RF: 0.75 kg AMS pellet/d for wk 1, 0.5 kg AMS pellet/d for wk 2; n = 98), 3) reduced both feeding and milking (RB: n = 73), and 4) a control (C: n = 75) group. Non-reduced milking allowed up to 6 milkings/d or as often as a cow was expected to yield 6 kg/milking. Non-reduced feeding allocated up to 2 kg/d of AMS pellet. From the AMS software, feed and milking behavior data were collected, as well as milk yield and SCC. Data on milk yield, milking frequency, and SCC were analyzed using mixed-effect linear regression models. The RB group had the lowest milk yield 3 d before dry-off, and was different from the C group (19.3 vs. 22.4 kg/d; SE = 1.08; $P = 0.01$). The RB group also differed from the C group in their reduction in total milk yield over the 2-wk treatment period (−4.9 vs. −1.8 kg; SE = 0.91; $P = 0.02$), indicating that this was the most efficient way to decrease milk yield before dry-off. Milking frequency was greater (SE = 0.09; $P < 0.001$) in the RF (2.25 ×/d) and C (2.65 ×/d) groups compared with the RM (1.60 ×/d) and RB (1.51 ×/d) groups. There was a difference ($P < 0.001$) in milking frequency between the RF and C groups, indicating that reducing feeding without altering milking frequency before dry-off may also decrease the motivation for cows to visit the AMS. There were no differences between groups ($P > 0.24$) for milking frequency or yield in the next lactation. SCC was not different ($P > 0.35$) between groups in the week before dry-off nor in the first month after calving. Overall, these data suggest that reducing both milking frequency and feed quantity in the AMS is the most efficient method to decrease milk yield before dry-off, without negatively influencing milking frequency or yield in the next lactation, as well as without affecting SCC.

Key Words: dry-off, robotic milking, udder health

391 Cow health and dairy farmer mental health in herds with robotic milking systems.

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The objective of this study was to survey dairy farmers using robotic milking systems to better understand their mental health and the potential connections to cow health. Of 76 farms in Ontario, Canada visited to survey management, cow health, and milk production, 34 farmers completed an online survey that included validated psychometric scales used to assess stress, anxiety, and depression. Thirty cows/farm (or 30% for herds > 100 cows) were scored for body condition (5-pt scale, 1 = thin to 5 = over-conditioned) and lameness (5-pt scale, 1 = sound to 5 = lame); cows with a BCS ≤ 2.5 and lameness score of ≥ 4 were defined as under-conditioned and severely lame, respectively. Univariable models were used to screen independent variables (as fixed effects) in mixed-effect linear regression models; variables with $P < 0.25$ were offered to multivariable models. At a farm level, the prevalence of severe lameness was correlated with average milk yield per cow, SCC, and the proportion under-conditioned cows; thus, only lameness prevalence was offered to multivariable models. Farmer stress was greater for females vs. males ($P = 0.007$), for those feeding manually vs. using an automated feeder (i.e., conveyer or automated delivery system; $P = 0.01$), and for those with a greater lameness prevalence ($P = 0.02$). Depression was greatest for those working alone on the farm ($P = 0.02$), those feeding manually ($P = 0.03$), and those with lesser average milk protein % ($P = 0.003$). Anxiety was greater for females vs. males ($P < 0.001$), and for those feeding manually ($P = 0.005$), working alone on the farm ($P = 0.07$), and with a greater lameness prevalence ($P = 0.02$) and lesser milk protein % ($P = 0.07$). Comparing our results to a similar survey of all commodity groups across Canada, dairy farmers using robotic milking systems may be experiencing less stress, anxiety, and depression than other farmers. The results highlight the potential benefits of automated milking and feeding systems and the difficulties associated with working alone. Farmer mental health was identified to be associated with milk yield, quality, and composition, in addition to cow health.

Key Words: automated milking, mental health, cow health

Forages and Pastures

392 The evaluation of the potential of biomass sorghum silage added with sugarcane. D. E. P. Oliveira[1], F. J. Ferreira[1], G. M. Dallago*[2], C. S. Bonfá[1], and M. A. Magalhães[1], [1]*Universidade Federal dos Vales do Jequitinhonha e Mucuri*, *Diamantina, Minas Gerais, Brazil*, [2]*McGill University*, *Sainte-Anne-de-Bellevue, Quebec, Canada*.

The seasonal production of forage is one of the main factors limiting animal performance in pasture-based systems under tropical climate conditions. It requires the search for alternative feedstuffs to offer animals during periods of forage scarcity to maximize animal performance. The objective of this study was to evaluate parameters of bromatological quality of silage of 3 genotypes of sorghum biomass (B012, B017, and B018) with 4 levels of sugarcane inclusion (0, 20, 40, and 60%). The experiment was conducted using a completely randomized design in a factorial scheme with 4 repetitions. The material was ensiled using PVC silos (10 cm diameter by 45 cm long) that were kept closed for 60 d. Upon opening, dry matter (DM), ash content (AC), organic matter (OM), and pH were measured. The data were analyzed using a 2-way ANOVA followed by the Tukey test ($\alpha < 0.05$). The silage of the genotype B012 had a higher ($P < 0.05$) DM (mean ± standard deviation = 29.3 ± 1.90) compared with the other genotypes (B017 = 27.3 ± 1.54 and B018 = 26.4 ± 1.32). We observed a linear negative effect ($P < 0.05$) of sugarcane on DM content, in which a reduction of 0.30% of DM was observed for each 10% increase in sugarcane inclusion. We observed an interaction effect ($P < 0.05$) between genotypes and sugarcane levels for OM and AC. There was no effect of sugarcane levels on OM and AC of the B012 genotype silage ($P > 0.05$). A quadratic positive effect of sugarcane levels was observed on OM of the silages of the genotypes B017 and B018 ($P < 0.05$), in which the lowest values were observed at 7.63% and 1.29% inclusion of sugarcane respectively. A quadratic negative effect was observed on AC of the same genotypes ($P < 0.05$), in which the highest values were observed at the same sugarcane level inclusion as for OM. The silage of the genotype B012 had a higher ($P < 0.05$) pH (4.26 ± 0.07) than the other genotypes (B017 = 4.14 ± 0.04 and B018 = 4.17 ± 0.06). The genotypes of biomass sorghum evaluated in our study responded satisfactorily to the fermentation process, which could result in silages of good quality, but the inclusion of sugarcane is not recommended.

Key Words: alternative feed, scarcity, ruminants

393 Prediction of compressed sward height of Walloon pastures from sentinel-2 images using machine learning algorithms. C. Nickmilder*[1], A. Tedde[1], P. Lejeune[1], I. Dufrasne[2], F. Lessire[3], B. Tychon[4], F. Lebeau[1], and H. Soyeurt[1], [1]*TERRA, ULiege*, *Liege, Belgium*, [2]*Departement de gestion veterinaire des Ressources Animales (DRA) / Nutrition des animaux domestiques, ULiege*, *Liege, Belgium*, [3]*Fundamental and Applied Research for Animals and Health (FARAH), ULiege*, *Liege, Belgium*, [4]*Departement des sciences et gestion de l environnement (Arlon Campus Environnement), ULiege*, *Liege, Belgium*.

ROADSTEP is a Walloon research program aiming to develop decision tools to help farmers in their daily herd monitoring on pastures. One of the aims is to develop a modeling tool to predict the availability of pasture feeding based on satellite images, meteorological variables and soil characteristics. 7737 compressed sward heights (CSH) were measured on 2 farms recorded with Jenquip EC20G platemeter in July and August 2019. They were used to calibrate and validate 73 predictive models of CSH. The tested algorithms were linear regression, lars, cubist, generalized linear model, neural network, random forest and linear support vector machine. The explaining variables were the 11 sentinel-2 reflectance bands at the bottom of atmosphere. Those bands and CSH were introduced directly in the model but also through their logarithm, square-root, square and cube forms to test the possible nonlinear relationships between them. The reduction of dimensionality of X-matrix through the estimation of principal components as well as partial least squares factors was also tested. To guarantee independence between calibration and validation, calibration was made on CSH (ranging from 12 to 158 mm with an average value of 59.4+-22.3 mm) measured on a farm and validation on CSH (ranging from 13 to 247.5 mm with an average value of 53.2+-21.6 mm) measured on another farm. The model that performed the best was a generalized linear model from the gamma family using an inverse link function. Calibration and validation RMSE were respectively equal to 17.4 and 20.7 mm or 29.3 and 28.9% of their respective mean. These results are only preliminary. Additional sampling periods and pastures are needed to improve the models' robustness. Moreover, the second step of this research will consist in adding information related to meteorological data and soil characteristics to enhance the prediction power of the developed models.

Key Words: remote sensing, compressed sward height, machine learning

394 Effects of diversity and spatial separation of pastures on milk yields, N partitioning, and methane emissions in dairy cows. L. Carmona-Flores*[1], M. Bionaz[1], T. Downing[1], M. Sahin[2], and S. Ates[1], [1]*Department of Animal and Rangeland Sciences, Oregon State University*, *Corvallis, OR*, [2]*Department of Crop and Soil Sciences, Oregon State University*, *Corvallis, OR*.

Diverse pastures containing multiple species help extend the grazing season and reduce the reliance on one or two species to meet all the nutritional requirements of livestock. Planting pasture species in spatially separated adjacent strips can potentially increase the dry matter intake (DMI) of high-quality forages leading to superior animal production. Thus, in the current study, combinations of simple and diverse pasture mixtures in mixed and spatially separated pasture strips were evaluated for their effects on feed intake, milk yield, N partitioning and methane emission of dairy cows. A 7.2-ha paddock was divided into 3 2.4-ha blocks to serve as replicates for the experiment. For the purpose, 36 mid-lactation Jersey cows were used in a randomized complete block design with 9 cows in each pasture (3 blocks each) as follow: 1)simple mixed (perennial ryegrass and white clover); 2)simple spatially separated; 3)diverse mixed (perennial ryegrass, festulolium, white clover, birdsfoot trefoil, plantain and chicory; 4)diverse spatially separated. Milk yield and quality, N partitioning, and methane emission were measured. Data were analyzed by ANOVA based on a 2 × 2 factorial model with significance declared at $P \leq 0.05$. Cows that grazed diverse pastures had greater milk solids (2310 g/d; $P = 0.05$) and milk protein yields (883 g/d; $P = 0.01$) as compared with those that grazed simple pastures (2083 g/d and 778 g/d, respectively). Spatial separation did not affect DMI, milk yield, or milk components except lactose content of milk, which was lower ($P = 0.01$) in spatially separated pastures. Although pasture diversity did not affect daily methane production, cows that grazed diverse pastures had lower ($P = 0.05$) methane yields per DM eaten (18.8 g) as compared with simple pastures (22.3 g). Cows that grazed diverse pastures had lower ($P = 0.01$) urine N (%) (0.3%) and urea content (104.1 mmol/L) and lower daily N output through urine (144.2 g/d). In conclusion, the diverse pastures containing species with certain agronomic and nutritional traits such as secondary metabolites can help improve animal production while decreasing the environmental effect of dairy farming.

Key Words: species diversity, methane emissions, N partitioning

395 Milk production, nitrogen utilization, and methane emission of dairy cows grazing grass, forb, and legume-based pastures. R. Wilson[1], M. Bionaz[1], J. MacAdam[2], K. Beauchemin[3], H. Naumann[4], and S. Ates*[1], [1]*Department of Animal and Rangeland Sciences, Oregon State University*, *Corvallis, OR*, [2]*Department of Plants, Soils, and Climate, Utah State University*, *Logan, UT*, [3]*Lethbridge Research and Development Centre, Agriculture and Agri-Food*, *Lethbridge, AB, Can-*

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Achieving high animal productivity without degrading the resource base is the primary target in pasture based-dairy farming. This study investigated the effects of changing the forage base in spring from grass-clover pastures to forb or legume-based pastures on milk yield, N partitioning and methane emissions of Jersey cows in Western Oregon. Twenty-seven mid-lactation dairy cows were randomly assigned to 1 of 3 pasture treatments: grass-clover based pasture composed of festulolium, soft-leaf tall fescue, orchardgrass, and white clover (Grass); forb-based pasture composed of chicory, plantain, and white clover (Forb); and legume-based pasture composed of red clover, birdsfoot trefoil, berseem clover and balansa clover (Legume). Pasture were arranged in a randomized complete design with 3 replicates. Production and nutritive quality of the forages, animal performance, nitrogen partitioning, and methane emissions were measured. Data were analyzed by ANOVA with pasture as main effect and plot as random (n = 3). Significance was declared with $P \leq 0.05$. Herbage production, feed quality, and DMI of cows were greater in Legume and Forb vs. Grass, with consequent larger milk yield and milk solid concentration. Cows grazing Forb also had higher lactose in milk compared with the other pastures, and lower somatic cell counts compared with Grass. Cow grazing Forb had substantially lower N in urine compared with cows grazing the other pastures but, together with cows grazed on Legume, larger fecal N. Both Forb and Legume had a diuretic effect on cows, as indicated by the lower creatinine concentration in urine but larger proportion of allantoin:creatinine and a tendency ($P = 0.08$) for greater microbial N production. Methane emissions tended ($P = 0.07$) to be lower in cows grazed on Forb vs. the other pastures. The results indicated a potential for legume- and forb-based pastures to fulfill nutritional deficiencies in late spring resulting in improved animal performance and health and reduced environmental impact of pasture-based dairy production.

Key Words: pasture systems, nitrogen partitioning, methane emission

396 Ruminal metabolism and plasma amino acids in Jersey cows grazing forage canola. L. H. P. Silva*[1], Y. Zang[1], M. Ghelichkhan[1], Y. Geng[2], S. L. Dillard[3], K. J. Soder[4], and A. F. Brito[1], [1]*University of New Hampshire*, *Durham, NH*, [2]*Chinese Academy of Agricultural Sciences*, *Beijing, China*, [3]*Auburn University*, *Auburn, AL*, [4]*USDA-Agricultural Research Service, Pasture Systems and Watershed Management Research Unit*, *University Park, PA*.

Forage canola is known for high biomass yield and exceptional supply of rapidly digestible carbohydrates, which can alter ruminal fermentation profile. It is also used to extend the fall grazing season. We aimed to evaluate the effect of canola as grazed herbage on ruminal fermentation and plasma AA in lactating dairy cows. Twelve multiparous and 6 primiparous mid-lactating Jersey cows were blocked by parity and, within block, assigned to control (CTRL) or canola (CAN) in a randomized complete block design. Cows in the CTRL group were kept in confinement, while CAN cows stayed in the barn during the day and had access to pasture from 1800 to 0500 h. Diets were formulated to yield a 60:40 forage:concentrate ratio with 50% of the baleage replaced by canola herbage in the CAN diet. The experiment lasted 7 wk (2-wk covariate) with sample collection done during wk-3 and wk-5. Data were analyzed with repeated measures in SAS. Daily herbage allowance was set at 12 kg of DM/cow, and ruminal fluid was collected using a stomach tube. Canola biomass averaged 4,000 kg of DM/ha, and 24.8% CP, 44.3% NFC, and 15.5% aNDFom. Diets did not affect ECM (mean = 29.2 kg/d) and urinary excretion of purine derivatives. Diet by wk interactions were found for PUN and ruminal NH_3-N showing that CAN cows had greater values at wk-5 than wk-3, but no change was observed for the CRTL diet. Ruminal pH was lower ($P < 0.01$) in CAN, while total VFA concentration did not change. Cows in the CAN diet had greater ruminal propionate than those in the CTRL (16.2 vs 13.5%, $P < 0.01$), possibly because of increased NFC and less aNDFom in canola herbage vs. baleage. The acetate:propionate ratio was lower in CAN than CTRL (4.21 vs. 5.61; $P < 0.01$). Significant diet by wk interactions were found for ruminal acetate and butyrate showing that acetate decreased while butyrate increased from wk-3 to wk-5; however, these changes were greater in CAN than CTRL diet. Individual AA in plasma was not affected by diet, except tryptophan which was lower in CAN. Diet did not affect plasma total essential AA. In brief, canola herbage improved ruminal propionate but did not affect plasma concentration of EAA.

Key Words: brassica, grazing, ruminal fermentation

397 Chicory and plantain-dominated forb pasture improves health and rumen N efficiency in lactating dairy cows. H. Ford*[1], S. Busato[1], E. Trevisi[2], Y. Gultekin[1], M. Bionaz[1], and S. Ates[1], [1]*Oregon State University*, *Corvallis, OR*, [2]*Università Cattolica del Sacro Cuore*, *Piacenza, Italy*.

Alternative pasture species like chicory and plantain are known to have high amounts of secondary metabolites that may mitigate N losses and inefficiencies, but their overall impact on animal health remains to be fully understood. The objective of this study was to identify the effects on health of dairy cows of chicory+plantain-dominated pasture using 26 blood parameters associated with metabolism and oxidative and immune status. For this purpose, 27 mid-lactation dairy cows were randomly assigned to one of the following pastures (3 blocks each): grass-clover (G, festulolium, tall fescue, orchardgrass and white clover), legume (L; red clover, birdsfoot trefoil, balansa clover and berseem clover), and forb (F; chicory, plantain and white clover). Cows grazed for 39 d over 2 periods. Blood was collected following morning milking at 7 time points over the 2 periods. Twenty-six parameters were assessed in plasma and data were analyzed using GLIMMIX with pasture as the main effect and block (n = 3) as random effect. Compared with G, cows grazed in both F and L pastures had significantly lower ($P < 0.05$) BHBA (0.45(F) and 0.51(L) vs. 0.59(G) mM) and creatinine (63.9(F) and 64.7(L) vs. 69(G) mM) and higher vitamin E (12.7(F) and 12.8(L) vs. 8.8(G) μg/mL) and FRAP (213.3(F) and 216.8(L) vs. 201.8(G)), supporting an effect on the microbiome, a diuretic effect, and improved antioxidative status. Compared with both G and L, cows in F pasture had significantly lower ($P < 0.05$) urea (2.9(F) vs. 4.4(G) and 7.4(L) mM) and urea:creatinine ratio (45.8(F) vs. 64.7(G) and 113.9(L)), indicating improved utilization of urea in the rumen. Compared with both G and F, cows in L pasture had significantly higher ($P < 0.05$) hematocrit, bilirubin, cholesterol, albumin, β-carotene, retinol, and thiol groups but lower ceruloplasmin, paraoxonase, and myeloperoxidase, indicating a positive health effect of L pasture on liver, inflammation, and red blood cells. Overall, these results indicate that chicory-plantain and legume pastures rich in secondary metabolites can improve animal health, and efficiency of N utilization, compared with the typical grass pasture.

Key Words: pasture, dairy, metabolites

Physiology and Endocrinology

398 Effects of LPS administration and subsequent nutrient restriction on metabolism in lactating dairy cows. S. Rodriguez-Jimenez*, E. A. Horst, E. J. Mayorga, M. A. Abeyta, B. M. Goetz, S. Carta, and L. H. Baumgard, *Iowa State University*, *Ames, IA*.

Objectives were to evaluate the effects of repeated LPS exposure and subsequent feed restriction (FR) on metabolism in lactating cows. Cows (631 ± 16 kg BW; 124 ± 15 DIM) were enrolled in a study consisting of 3 experimental periods (P); during P1 (5d) cows were ad libitum-fed and baseline data were obtained. During P2 (7d), cows were assigned to 1 of 2 treatments: 1) saline-infused and pair-fed (PFFR; 5 mL saline on d1, 3, and 5; n = 6) or 2) LPS-infused and ad libitum-fed (LPSFR; 0.2, 0.8, and 1.6 μg LPS/kg BW on d1, 3, and 5, respectively; n = 6). During P3 (7d), all cows were feed restricted to 50% of P1 feed intake. On d3 of P3, LPSFR cows received an LPS bolus (0.8 μg LPS/kg BW) whereas PFFR cows received saline. Blood samples were obtained on d 1 and 3 of P1, and on d 1, 3, 5, and 7 of P2 and P3. Ketone tolerance tests (KTT) were performed on d3 of P1, and on d 2 and 6 of P2 and P3. Data were analyzed using PROC MIXED of SAS. Relative to P1, insulin decreased in both treatments during P2 and P3 ($P < 0.01$). Relative to PFFR, insulin from LPSFR increased overall during P2 (2.5-fold; $P < 0.01$) and transiently on d3 of P3 (2.6-fold; $P < 0.01$). Circulating NEFA from PFFR increased markedly from d 1 to 3 of P2 (3.4-fold relative to P1; $P < 0.01$) and then slowly decreased, whereas levels remained unchanged in LPSFR related to P1. Overall in P2, circulating NEFA from PFFR cows increased (63%) relative to LPSFR ($P < 0.01$). During P3, NEFA levels did not differ across treatments but were increased in both PFFR and LPSFR cows relative to P1 ($P < 0.01$). No treatment differences were detected for circulating BHB during P2, however, concentrations increased (18%) on d 5 of P3 in PFFR cows while in LPSFR remained similar ($P = 0.07$) relative to P1. In response to the KTT, BHB area under the curve decreased in LPSFR relative to PFFR cows on d 2 and 6 of both P2 and P3 (30, 47, 30, and 13% respectively; $P < 0.01$). In conclusion, inflammation caused hyperinsulinemia and increased BHB clearance rates.

Key Words: immune activation, ketone tolerance test

399 Response to LPS challenge after intravenous amino acid infusion in postpartum dairy cows. T. L. Chandler*, T. A. Westhoff, C. R. Seely, J. E. Cha, M. E. Van Amburgh, T. R. Overton, and S. Mann, *Cornell University*, *Ithaca, NY*.

Postpartum cows experience a heightened inflammatory state coinciding with energy and amino acid (AA) deficit. Our objective was to describe the response to a systemic inflammatory stimulus after a 4-d intravenous (IV) AA infusion designed to fill the cows' estimated MP deficit. Cows (n = 14, 4 ± 1 DIM) were IV infused 24 h/d for 4 d in a matched-pair randomized controlled design and received IV AA (IVAA) or 0.9% NaCl (CTRL). Daily IVAA were infused at 10 mL/kg BW to supply 1 g/kg BW of essential AA (EAA) and nonessential AA (NEAA). After infusion ended, cows were IV infused with LPS (0.0625 μg/kg BW over 1 h) and blood samples were collected at 0, 1, 2, 3, 4, 8, and 24 h for analysis of glucose, nonesterified fatty acids (NEFA), insulin, glucagon, and AA. Repeated measures ANOVA in PROC MIXED (SAS v. 9.4) included fixed effects of time, treatment (trt), time × trt, DIM, parity, and BW, and random effect of pair. Glucose was similar ($P > 0.15$) between groups at 0 h (61 vs. 67 ± 4 mg/dL, IVAA vs. CTRL ± SEM) and decreased over time ($P = 0.01$) reaching a nadir at 2 h, but was lower ($P < 0.01$) in IVAA over the 24 h (55 vs. 64 ± 3 mg/dL). Plasma NEFA were greater ($P < 0.01$) in IVAA following LPS [673 (473–957) vs. 426 (313–579) μeq/L, IVAA (95% CI) vs. CTRL (95% CI)], but changed over time ($P = 0.02$) in both groups (time × trt $P = 0.21$). Glucagon was greater ($P < 0.01$) in IVAA following LPS [242 (200–294) vs. 171 (142–204) pg/mL] and was similarly affected by time ($P < 0.01$) in both groups (time × trt $P = 0.53$). Insulin was lower ($P < 0.01$) in IVAA following LPS [3.1 (2.5–3.9) vs. 4.8 (4.0–5.8) μIU/mL] and decreased over time ($P < 0.01$) in both groups (time × trt $P = 0.94$). Plasma EAA (1479 vs. 884 ± 83 μ*M*), NEAA (1677 vs. 1361 ± 85 μ*M*), and branched-chain AA (BCAA) (1008 vs. 545 ± 66 μ*M*) were greater ($P < 0.01$) in IVAA vs. CTRL at 0 h. Plasma EAA and BCAA decreased over time, reaching a nadir at 8 h, but the effect depended on trt (time × trt $P < 0.01$). Amino acid infusion did not appear to affect the time-dependent metabolic response to LPS, given the lack of time × trt interactions; however, AA responses were characterized by greater decreases in IVAA over time.

Key Words: transition period, inflammation, glucagon

400 Intravenous amino acid infusion in early postpartum dairy cows: Effects on performance, blood metabolites, and serum hormones. T. L. Chandler*[1], T. A. Westhoff[1], J. E. Cha[1], A. L. Lock[2], T. R. Overton[1], and S. Mann[1], [1]*Cornell University*, *Ithaca, NY*, [2]*Michigan State University*, *East Lansing, MI*.

Early postpartum cows are deficient in amino acids (AA). Production responses to metabolizable protein (MP) infused abomasally, notably milk protein % or yield, have been observed. Our objective was to describe the response to an intravenous (IV) AA infusion designed to fill the estimated MP deficit in postpartum cows. Cows (n = 14, 4 ± 1 DIM) were infused IV 24 h/d for 4 d in a matched-pair randomized controlled design and received IV AA (IVAA) or 0.9% NaCl (CTRL). Daily IVAA were infused at 10 mL/kg BW to supply 1 g/kg BW of essential and nonessential AA. Cows were fed TMR ad libitum and milked 2 × daily. Milk samples taken at each milking were analyzed and composited for milk components and milk fatty acid (FA) profile. Daily blood samples were collected before feeding and analyzed for glucose, nonesterified FA (NEFA), triglyceride (TG), insulin, and glucagon. Repeated measures ANOVA with baseline covariates were analyzed by PROC MIXED (SAS v. 9.4) with fixed effects of time, treatment (trt), time × trt, DIM, parity, and BW, and random effect of pair. Results are summarized in Table 1. Amino acid infusion limited DMI, but increased milk fat % and yield. Milk protein was unaffected. Amino acid infusion was associated with increased circulating glucagon, NEFA, and TG. Increased milk fat originated from an increase in both de novo and preformed FA. Given the effect of IVAA on TG and NEFA the source of preformed FA is of interest and should be investigated further.

Key Words: transition period, fatty acid, glucagon

401 The effect of manual forestripping on teat tissue condition and milking performance in Holstein dairy cows milked 3 times daily. M. Wieland*[1], P. D. Virkler[1], A. Weld[1], J. M. Melvin[1], M. R. Wettstein[1], M. F. Oswald[1], C. G. Geary[1], R. D. Watters[1], R. Lynch[2], and D. V. Nydam[1], [1]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University*, *Ithaca, NY*, [2]*Department of Animal Science, College of Agriculture and Life Sciences, Cornell University*, *Ithaca, NY*.

The objectives were to study the effect of manual forestripping on (1) teat tissue condition and (2) milking characteristics. In a randomized controlled crossover study, 130 Holstein dairy cows were assigned into treatment and control groups. Premilking udder preparation for the treatment group consisted of: step 1, predipping with 1% iodine; step 2, forestripping of 3 streams of milk per quarter and wiping of teats; and step 3, attachment of the milking unit. Premilking udder preparation for the control group was identical except forestripping was not performed. The time spent from first tactile stimulus to milking unit attachment was kept consistent at 90 s for both groups. The study lasted for 14 d with 2 periods, each consisting of a 2 d adjustment time followed by 5 d of data collection. Machine milking induced short-term changes to the teat tissue (STC) were assessed visually. Milking characteristics [milk yield (kg;

Table 1 (Abstr. 400).

				P-value		
Item	CTRL (n=7)	AA (n=7)	SEM	Trt	Time	Time×trt
DMI, kg/d	20.0	18.2	0.6	0.01	0.07	0.03
Milk, kg/d	35.1	35.2	1.1	0.91	<0.01	0.39
Fat, %	4.93	5.85	0.09	<0.01	<0.01	<0.01
Fat yield, g/d	1720	2029	50	<0.01	<0.01	<0.01
de novo FA, g/d	314	352	10	0.02	<0.01	0.13
C16 FA, g/d	574	637	27	0.10	<0.01	0.01
Preformed FA, g/d	723	825	10	<0.01	<0.01	0.09
Protein, %	3.74	3.67	0.06	0.35	<0.01	0.03
Protein yield, g/d	1305	1266	45	0.29	0.03	0.84
ECM, kg/d	43.3	46.9	1.3	<0.01	<0.01	0.09
Glucose, mg/dL	68.8	65.0	3.0	0.33	0.80	0.87
NEFA, µEq/L (95% CI)	512 (344–762)	893 (549–1,451)		0.07	0.94	0.46
TG, mg/dL	8.2	10.4	1.0	0.10	0.69	0.13
Insulin, µIU/mL (95% CI)	4.5 (3.0–6.7)	3.2 (1.9–5.2)		0.21	0.45	0.97
Glucagon, pg/mL	141	188	11	<0.01	<0.01	0.06

MY), milking unit on time (s; MUOT), 2 min milk yield (kg; 2MIN), and time spent in low milk flow rate (s; LMF)], were assessed with electronic on-farm milk meters. Generalized linear mixed models were used to study the effect of treatment on the outcome variables. The odds of STC were lower for cows that received forestripping compared with cows that were not forestripped [odds ratio (95% confidence interval, 95% CI) = 0.31 (0.22–0.42)]. Least squares means (95% CI) for cows that were forestripped and animals that were not forestripped, respectively, were MY, 12.71 (12.24–13.19) and 12.69 (12.22–13.16) kg; MUOT, 253 (245–261) and 263 (254–271) s; 2MIN, 6.11 (5.84–6.38) and 5.59 (5.31–5.86) kg; and LMF, 16 (15–17) and 19 (18–21) s. In this study, cows that were forestripped had higher 2MIN, shorter MUOT, shorter LMF, and lower odds of STC. We conclude that wiping of teats during premilking udder preparation may not provide sufficient tactile stimulation to elicit the cows' maximum physiological milk ejection capacity. This can aggravate the adverse effects of machine milking to the teat tissue, diminish animal well-being, and possibly impact udder health.

Key Words: milking routine, oxytocin, animal welfare

402 Effect of human chorionic gonadotropin treatment on d 7 or d 7 and 13 of the estrous cycle on luteal and follicular dynamics in non-inseminated lactating Holstein cows. T. O. Cunha*[1], L. R. Statz[1], R. R. Domingues[2], J. P. N. Andrade[1,2], M. C. Wiltbank[2], and J. P. N. Martins[1], [1]*School of Veterinary Medicine, University of Wisconsin-Madison*, *Madison, WI*, [2]*Department of Dairy Science, University of Wisconsin-Madison*, *Madison, WI.*

Our objective was to determine the effect of human chorionic gonadotropin (hCG; 3,300 IU i.m.) on d 7 (hCG7) or d 7 and 13 (hCG13) of the estrous cycle on ovarian function in lactating Holstein cows. Cows (44.2 ± 0.6 DIM) were pre-treated with an Ovsynch+CIDR protocol, and only synchronized cows were used (n = 64). The day of the last GnRH was considered d0 of the estrous cycle. Follicular and luteal dynamics of cows were evaluated daily during an entire estrous cycle by ultrasound. Blood samples were collected daily to measure serum concentration of progesterone (P4). Cows were randomly assigned to CON (n = 22, no treatment), hCG7 (n = 20), or hCG13 (n = 22) treatments. Only cows that ovulated by 2d after hCG treatments were used in the study (n = 62). The first day of luteolysis was considered the day that P4 declined to < than 2x the SD of the mean for the 4 highest P4 levels in late diestrus. The P4 cut-off for complete luteolysis was <1.0 ng/mL. Mean P4 (3.20 ± 0.16 ng/mL) did not differ (*P* = 0.48) between treatments on d7. Cows treated with hCG had greater (*P* < 0.01) P4 between d 8 and 15 of the cycle than CON. Cows treated with hCG13 had greater (*P* < 0.02) P4 on d 14 and 15 of the cycle than hCG7. Cows were classified as atypical if the dominant follicle (DF) or future DF at the time of luteolysis did not ovulate (CON, n = 2; hCG7, n = 4; hCG13, n = 3), had a short cycle (CON, n = 1), delayed (CON, n = 2) or incomplete luteolysis (CON, n = 1; hCG7, n = 4; hCG13, n = 5). Most cows with incomplete luteolysis (n = 9/10) had at least 2 ipsilateral CL in one ovary, and 1 or 2 CL did not regress. Proportion of cows with typical cycle were 73% (16/22) for CON, 60% (12/20) for hCG7, and 60% (12/20) for hCG13. Typical cycle cows treated with hCG had delayed (*P* ≤ 0.03) luteolysis, prolonged time (*P* < 0.01) to undergo complete luteolysis, and more cows with 3 follicular waves (*P* < 0.01; hCG7 = 92%; hCG13 = 100%; CON = 25%) than CON; resulting in a longer interovulatory interval (*P* < 0.01) for hCG7 (24.9 d ± 0.4) and hCG13 (26.0 d ± 0.7) than CON (22.0 d ± 0.5). In summary, hCG treatments had an effect not only on the follicular and luteal dynamics but also on the luteolysis process.

Key Words: human chorionic gonadotropin (hCG), corpus luteum, luteolysis

Production, Management, and the Environment

403 Characterizing the microbiota of recycled bedding sand on a Wisconsin dairy farm. H. Pilch[1], A. Steinberger*[2], G. Suen[2], N. Aulik[3], D. Sockett[3], and C. Czuprynski[1], [1]*Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI*, [2]*Department of Bacteriology, University of Wisconsin-Madison, Madison, WI*, [3]*Wisconsin Veterinary Diagnostic Laboratory, Madison, WI.*

Recycling bedding sand is a common practice on dairy farm operations. Little is known about the diverse microbial community in bedding sand and how it might impact dairy cattle health. Here, the microbiota of recycled bedding sand on a WI dairy farm was evaluated at various stages of the recycling process across 2 seasons. Samples of sand and gray water were collected from several locations on a Wisconsin dairy farm using sterile wooden spoons and conical tubes, once during the summer and winter of 2018. DNA was extracted from each sample and the V4 variable region of 16S rRNA genes were PCR amplified. Amplicons were sequenced on an Illumina MiSeq, processed using the mothur software package, and analyzed with R. A total of 5,064 unique operational taxonomic units (OTUs) representing 32 phyla, 289 families, and 562 genera were identified in summer samples. A core microbiota of 171 OTUs were present across all summer sampling locations suggesting persistence of bacteria across the recycling process. Bacterial community composition (BCC) of the summer samples also differed by location across the sand recycling process ($P < 0.05$). In winter, a total of 3,158 unique OTUs representing 28 phyla, 294 families, and 663 genera were identified. A core group of 236 OTUs were shared across all winter locations. There was no relation between location and BCC for winter samples ($P > 0.05$). *Flavobacterium, Psychrobacter*, and *Pseudomonas*, genera that contain known environmental mastitis pathogens, were identified as the most abundant genera in recycled bedding sand in both winter and summer. *Enterococcus*, another mastitis pathogen, was also found in high relative abundance but only across winter samples. These data demonstrate that recycled sand bedding contain complex bacterial communities which persist, in part, across the sand recycling process during both summer and winter. Comparison of summer and winter samples further suggest a seasonal effect on the microbiota of recycled bedding sand and gray water. Together these findings suggest that recycled bedding sand could serve as a source of infection for dairy cattle, warranting further study.

Key Words: microbiome, recycled bedding sand, dairy farm

404 Effects of 2-hydroxy-(4-methylthio) butanoic acid and isoacids on rumen fermentation, production, and milk fatty acid during mild milk fat depression in lactating cows. J. E. Copelin*[1], J. L. Firkins[2], M. T. Socha[3], and C. Lee[1], [1]*Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH*, [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH*, [3]*Zinpro Corporation, Eden Prairie, MN.*

The objective of this experiment was to determine effects of 2-hydroxy-(4-methylthio) butanoic acid (HMTBa), isoacids (isobutyrate, isovalerate, 2-methylbutyrate, valerate) or their combination on alleviation of milk fat depression (MFD). Ten Holstein cows (5 cannulated and 5 non-cannulated) were used in a repeated 5 × 5 Latin square design. Treatments included 1) high forage diet (HF; 33% NDF, 24.0% starch, and 3.5% PUFA on a DM basis), 2) low forage diet (LF; 29% NDF, 29% starch, and 3.5% PUFA), 3) LF with HMTBa at 0.1% (DM basis; LF-HMTBa), 4) LF with isoacids (60 g/d; equal amounts of all isoacids; LF-IA) and LF supplemented with both HMTBa and IA. Data were analyzed using the Mixed procedure of SAS [random effect, square and cow(square); fixed effect, diet]. Preplanned contrasts were used to compare HF vs. LF and to examine the effect of HMTBa, IA, or their interaction compared with LF. Molar proportion of propionate increased ($P < 0.01$) and acetate decreased ($P = 0.10$) for LF vs. HF. Supplementation of HMTBa increased ($P = 0.04$) molar proportion of butyrate compared with LF. The addition of IA increased ($P < 0.01$) molar proportions of branched-chain VFA in the rumen. Milk fat content (3.20 vs. 3.46%; $P = 0.04$) and yield (0.86 vs. 0.98 kg/d; $P < 0.01$) decreased for LF vs. HF. The MFD with LF was alleviated by LF-HMTBa and LF-IA; HMTBa increased milk yield ($P = 0.03$) without altering milk fat content and LF-IA tended to increase ($P = 0.08$) milk fat content without altering milk yield. However, an interaction for milk fat yield ($P < 0.01$) was observed between HMTBa and IA, suggesting no additive effect. Moreover, LF-IA decreased ($P = 0.03$) total preformed fatty acids and increased ($P = 0.04$) de novo synthesized fatty acids compared with LF treatment. In conclusion, during mild MFD, supplementation of HMTBa or IA alleviated the MFD. However, the alleviation of MFD appeared to be a result of different mechanisms, and no additive effects of the combination were observed on milk fat yield.

Key Words: 2-hydroxy-(4-methylthio) butanoic acid (HMTBa), isoacids, milk fat depression

405 Successful strategies to reduce enteric methane emission from ruminants: A meta-analysis. C. Arndt*[1], A. N. Hristov[2], W. J. Price[3], S. C. McClelland[4], A. Pelaez[2], S. F. C. Welchez[2], J. Oh[5], A. Bannink[6], A. R. Bayat[7], L. A. Crompton[8], J. Dijkstra[6], M. A. Eugène[9], M. Kreuzer[10], M. McGee[11], C. Martin[12], [1]*Universidad Nacional Agraria, La Molina, Lima, Peru*, [2]*The Pennsylvania State University, University Park, PA*, [3]*University of Idaho, Moscow, ID*, [4]*Colorado State University, Fort Collins, CO*, [5]*Cargill Korea, Seoul, South Korea*, [6]*Wageningen University and Research, Wageningen, the Netherlands*, [7]*Natural Resources Institute Finland, Finland*, [8]*University of Reading, Reading, UK*, [9]*INRAE, Saint Genès-Champanelle, France*, [10]*University of California, Davis, CA*, [11]*ETH Zurich, Zürich, Switzerland*, [12]*Teagasc, AGRIC, Grange, Ireland*.

This meta-analysis examined the effects of mitigation strategies on enteric CH_4 emission (CH_4E, g/d), Y_m (CH_4 energy, % of gross energy intake), CH_4 yield [g/kg dry matter intake (DMI)], CH_4 emission intensity [Ei, g/kg milk yield (MY) or average daily gain (ADG)], DMI (kg/d), ADG (kg/d), MY (kg/d), and neutral detergent fiber digestibility (NDFD, %) in ruminants. The database consisted of data reported in 437 published studies (1963 to 2018) using cattle (65% of the data) and small and other ruminants (35%). Mitigation strategies were classified into 3 main categories: animal and feed management, diet formulation, and rumen manipulation, and up to 5 subcategories (99 total mitigation strategy combinations, which are not discussed here). A random-effects meta-analysis weighted by inverse variance was carried out (Comprehensive Meta-Analysis, V3.3.070). Mitigation effects were based on the relative mean ratio (treatment over control) to standardize effects across studies. Significance was based on $\alpha = 0.05$ with values adjusted for multiple comparisons. Daily CH_4 emissions were analyzed in 783 mean comparisons, followed by number of mean comparisons in descending order by DMI (706), CH_4 yield (598), ADG (376), Y_m (354), CH_4 Ei (260), MY (245), and NDFD (206). Rumen manipulation decreased ($P \leq 0.05$) CH_4E (−11%), Y_m (−10%), CH_4 yield (−12%), and CH_4 Ei (−12% for ADG) and increased ($P \leq 0.01$) DMI (+1%). Diet formulation decreased ($P \leq 0.01$) CH_4E (−7%), Y_m (−10%), CH_4 yield (−10%), and CH_4 Ei (−10% and −8% for ADG and MY, respectively) and increased ($P \leq 0.01$) DMI (+2%) and MY (+7%) but it also decreased NDFD (−3%; $P \leq 0.01$). Animal and feed management increased (+7%; $P \leq 0.01$) CH_4E, but it also increased ($P \leq 0.01$) DMI (+17%), ADG (+27%), MY (+11%), and NDFD (+6%) and thus decreased ($P \leq 0.01$) Y_m (−7%) and CH_4 Ei (−10% for MY). Specific practices within these main mitigation strategies effectively decreased CH_4 emission without compromising animal productivity. The practical implementation of mitigation strategies will depend on proven long-term effects, economic feasibility, government policies, and consumer acceptance.

Key Words: enteric methane, mitigation, meta-analysis

406 Altering the ruminal microbiota in dairy calves using rumen contents dosing. M. Cox*[1], P. Weimer[2,1], A. Steinberger[1], J. Skarlupka[1], and G. Suen[1], [1]*Department of Bacteriology, University of Wisconsin-Madison*, *Madison, WI*, [2]*US Dairy Forage Research Center, USDA Agricultural Research Service*, *Madison, WI.*

A major goal in dairy research is to improve milk production efficiency (MPE). With the advent of next-generation sequencing and its use in characterizing microbial communities, efforts are underway to improve MPE by manipulating the rumen microbiota. MPE is correlated with ruminal bacterial community composition (BCC), but the adult rumen microbiota is highly stable and returns to a baseline BCC even after heavy perturbation. We seek to influence rumen BCC by early intervention in pre-weaning dairy calves. Two cannulated Holstein donors of disparate MPE were selected. Three cohorts of 6 bull calves were established and dosed by gavage with a rumen inoculum sourced from the high-efficiency donor (HE), the low-efficiency donor (LE), or an autoclaved 50:50 mix as a microbe-free control (C). Dosing occurred within 3 d of birth, then every 2 weeks through 6 weeks of age. Feces were collected at each dosing as a proxy for gut BCC. Daily dry matter intake of calf starter, which has been shown to predict downstream feed efficiency, was greatest in HE calves and lowest in C calves ($P < 0.05$), though preweaning average daily gain did not differ between cohorts ($P = 0.210$). Calves were sacrificed at 8 weeks to access rumen contents and rumen wall sections were collected to assess papillation. Fecal and rumen samples were subjected to 16S rRNA amplicon sequencing. We found that BCC differed by cohort in fecal and rumen samples ($P < 0.05$), with HE calf samples most similar to adult rumen samples and C calves least similar. Additionally, HE calves tended to have elongated papillae ($P = 0.062$), the development of which is dependent on byproducts of microbial metabolism in the rumen and the long-term impact of which points to differences in absorptive capacity of the ruminal epithelium. These data demonstrate that the rumen BCC can be influenced by early intervention. Ongoing work includes expansion of this dosing protocol to a cohort of 60 female calves, following rumen BCC development and the impact on MPE in through the first lactation.

Key Words: rumen, calf, microbiota

407 Effects of *Asparagopsis taxiformis* and oregano leaves on methane emission, rumen fermentation, and lactational performance of dairy cows. H. A. Stefenoni*[1], S. E. Räisänen[1], S. F. Welchez[1], D. E. Wasson[1], C. F. Lage[1], A. Melgar[1], M. E. Fetter[1], M. Hennessy[2], B. Vecchiarelli[2], J. Bender[2], D. Pitta[2], C. Yarish[3], and A. N. Hristov[1], [1]*The Pennsylvania State University*, *University Park, PA*, [2]*University of Pennsylvania*, *Kennett Square, PA*, [3]*University of Connecticut*, *Storrs, CT.*

The objective of this experiment was to determine the effect of *Asparagopsis taxiformis* (AT) and oregano leaves (ORE) on enteric methane (CH$_4$) emission, rumen fermentation, and lactational performance of dairy cows. Twenty Holstein cows (±SD) [95 ± 22.0 d in milk and 42 ± 2.6 kg milk yield (MY)] were used in a replicated 4 × 4 Latin square design with 4, 28-d periods. Treatments were a basal diet (CON) and CON supplemented with 0.25% AT (LAT), 0.50% AT (HAT), or 2.0% ORE of dry matter intake (DMI). Enteric gas emissions were measured using the GreenFeed system and rumen samples were collected by the ororuminal technique. Data were analyzed using PROC MIXED of SAS with treatment and period in the model. Square and cow within square were random effects. Compared with CON, HAT decreased ($P < 0.001$) average daily CH$_4$ emission and CH$_4$ yield by 65% (380 and 131 g/d) and 55% (14.0 and 6.32 g/kg DMI), respectively, in experimental periods (P)1 and 2, but had no effect in P3 and P4. The differential response to HAT in P3 and 4 could be attributed to a decrease in bromoform concentration in AT over time (about 74% decrease in 5 mo of storage) observed in a separate experiment. ORE and LAT had no effect on CH$_4$ emission. Compared with CON, HAT decreased ($P \leq 0.006$) DMI, MY, and energy-corrected MY by 6.9, 5.7, and 7.3%, respectively. Milk fat and true protein concentrations were not affected by treatment, but lactose was decreased ($P < 0.001$) by HAT, compared with CON. Total VFA concentration and acetate:propionate ratio tended ($P = 0.06$) to be lowest for HAT. HAT and ORE increased ($P = 0.02$) the molar proportion of propionate compared with CON and LAT. Both AT treatments had greater concentrations of butyrate ($P < 0.001$) compared with ORE and CON. In this experiment, AT fed at 0.5% of DMI decreased CH$_4$ emission by ≥55% in P1 and P2 of the experiment but the effect disappeared by P3 and P4, most likely due to a decrease in bromoform concentration. HAT also decreased DMI and milk production. ORE had no effect on CH$_4$ emission and lactational performance of the cows.

Key Words: methane, *Asparagopsis taxiformis*, dairy cows

408 Survey of perceptions and practices of antimicrobial drug use in preweaned California dairy calves. E. Okello*[1,2], D. Williams[1], R. Pereira[2], T. Lehenbauer[1,2], and S. Aly[1,2], [1]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis*, *Tulare, CA*, [2]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis*, *Davis, CA*.

The California dairy industry was surveyed in July 2017 to evaluate producers' knowledge, perceptions and antimicrobial drug (AMD) use in preweaned dairy calves following the implementation of veterinary feed directive (VFD) changes in Jan 2017 and before the CA Senate Bill (SB) 27 effective Jan 2018. These regulations required veterinary oversight of medically important antimicrobial drugs (MIADs) administered to livestock. Questionnaires were mailed to 1,361 CA grade A dairies and calf ranches across CA and 169 (12%) responded. Most respondents (83%) were aware of the VFD and SB 27 changes. Use of antibiotics was perceived as important (77%) in raising preweaned dairy calves and judicious use of antibiotics was ranked as the most important antimicrobial stewardship practice (n = 134) among good record keeping, observing withdrawal periods, having a valid Veterinarian-Client-Patient-Relationship, and use of alternatives to antibiotics. Producers indicated that calves were exposed to AMD either directly through parenteral and oral dosage forms (78%) or indirectly through hospital milk (44%). Treating sick calves was the major indication for AMD use; however, few (12.7%) producers reported use of antibiotics to control or prevent disease (11%). Neomycin sulfate, chlortetracycline, oxytetracycline and sulfamethazine were the most used AMD, though only 32% of the respondents kept a drug inventory. Decreased use of AMD post-VFD was noted in milk (10%) and grain (5%) and reported treatment records included date (82%), dose (44%) and route (15%) of AMD used. Only 13% and 16% respondents noted a decrease or increase in AMD costs respectively. Whereas most producers had knowledge of the VFD and SB 27, opportunities exist to improve AMD use practices, including record keeping and using AMD alternatives. The limited changes noted in AMD use could be due to the short period between the implementation of VFD and the time of the survey. Results of this survey provide a baseline for future evaluation of the impact of these regulatory changes and guide future best practice recommendations to promote judicious use of AMD.

Key Words: antimicrobial drug use, producer perceptions, dairy calf

409 High-throughput phenotyping of rumen microbial contents using buccal swabs. J. Young[1], R. Tassinari[2], K. Kalscheur[1], J. McClure[1], P. Weimer[1,4], A. Fischer[3], G. Suen[4], and D. Bickhart*[1], [1]*USDA DFRC, Madison, WI*, [2]*Federal University of Goias*, *Goiania, Brazil*, [3]*Institute De L'elevage*, *Lyon, France*, [4]*University of Wisconsin*, *Madison, WI.*

The rumen microbial community is the agent that allows cattle and other ruminants to process complex plant polymers into volatile fatty acids. Traditional methods to sample rumen microbes are often labor-intensive, or require invasive surgeries to access the rumen lumen via cannula ports, thereby limiting the number of animals that could be sampled in a specific study. To statistically detect the effects of inter-animal variation that impacts rumen microbial composition, many hundreds of rumen samples would be required for each trial. In this study, we tested the viability of using buccal swabs as a proxy of the rumen microbial contents in a timecourse experiment on 8 cannulated cows. Rumen contents and buccal swabs were collected at 6 equally spaced time points, with the first time point being 2 h before feeding. Simpson diversity and Shannon evenness estimates of the microbial counts of each sample revealed that the first time point had the lowest diversity and highest evenness (Tukey HSD <0.05) out of all other time points. Principal component analysis confirmed that the buccal swab samples from the first time point were the most similar to paired rumen samples taken at the same times. Using a Random Forest Classifier analysis, we estimated the Gini importance scores for individual microbial taxa as a proxy of their uniqueness to the rumen or oral environments of the cows. Using these estimates, we identified 18 oral-only microbial taxa that are contaminants and could be removed from future comparisons using this method. Finally, we attempted to estimate the exact relative abundance of rumen microbial taxa from buccal swab samples using paired rumen-swab data in a Random Forest Regression model. The model was found to have moderate (~38%) accuracy in cross-validation studies, suggesting that there are other factors such as salivary flow that could impact microbial data from buccal swabs. Our data suggests that buccal swabs can serve as fast and suitable proxies for rumen microbial contents of dairy cattle, but that additional factors must be measured to improve direct regression of results to those of the rumen.

Key Words: rumen, microbiology, phenotyping

410 Differences in methanogenesis pathways and microbial diversity in the rumen of low- and high-methane-yield phenotype dairy cows. D. Pitta*[1], H. Stefenoni[2], N. Indugu[1], M. Hennessy[1], B. Vecchiarelli[1], V. Shabtai[1], C. Welchez[2], S. Räisänen[2], D. Wasson[2], A. Melgar[2], M. Fetter[2], and A. Hristov[2], [1]*University of Pennsylvania School of Veterinary Medicine*, *Kennett Square, PA*, [2]*The Pennsylvania State University*, *University Park, PA.*

The objective of this experiment was to identify differences in microbiota and methanogenesis pathways in the rumen of phenotypically low and high methane-yield cows. Following an initial screening period, 5 phenotypically low (LOW) and 5 phenotypically high (HIGH) methane-emitting cows, blocked by parity, days in milk, and milk yield, were used to investigate the functional role of rumen microbiota. All cows were adapted to a standard diet for 3 weeks and sampled for rumen contents at 0, 2, 4, 8, 12, 14, 16, and 20 h post-feeding using the ororuminal technique. The solid fraction of the ruminal samples was extracted for DNA, PCR-amplified for the bacteria and archaea 16S rDNA gene, sequenced and analyzed for bacteria and archaeal diversity. Additionally, the genomic DNA from 2h post-fed rumen samples was prepared for metagenomic analysis and genes were annotated against the KEGG database. Methane emissions (346 vs 439 ± 28.6 g/d) were lower ($P = 0.03$) for LOW vs. HIGH cows. At the community level, both unweighted and weighted UniFrac analysis revealed differences ($P < 0.05$) in bacteria and archaea communities between groups. Among methanogens, *Methanobrevibacter* was lower and *Methanosphaera* was higher ($P < 0.05$ for both) in LOW vs. HIGH groups. In bacterial DNA and cDNA, genera such as *Prevotellaceae* and *Succinovibrionaceae* were higher in the LOW group, while genera such as *Ruminococcaceae* were higher in the HIGH group. Among the methanogenesis pathways, across both groups, the CO_2/formate-reducing pathway was predominant followed by the methanol-reducing pathway. *Methanobrevibacter* and *Methanosphaera,* respectively, played significant roles in these pathways. The number of gene copies encoding the α, β, and γ chains of methyl co-enzyme reductase (MCR), an enzyme present in all methanogens that facilitates the formation of methane, was lower ($P < 0.05$) in LOW vs. HIGH cows (336 vs. 452 copies per million, respectively). It can be concluded that the 22% difference in methane emissions is accompanied by a 25% difference in gene copies coding for MCR enzyme between HIGH and LOW cows.

Key Words: metagenomics, bacteria-archaea, methyl co-enzyme reductase (MCR)

411 Experimental cases of rumen hypomotility. D. Cavallini*[1,2], F. Boffo[1], L. Mammi[1], G. Canestrari[1], S. Speroni[1], G. Buonaiuto[1], L. Campidonico[1], F. Ghiaccio[1], A. Palmonari[1], E. Valle[2], and A. Formigoni[1], [1]*Department of Veterinary Sciences, University of Bologna*, *Ozzano Emilia, Italy*, [2]*Department of Veterinary Sciences, University of Turin*, *Grugliasco, Italy*.

Cases of sudden drop in rumination time (RT) are commonly defined as rumen hypomotility (RH). These events are often due to different causes, such as environmental stress, digestive pathologies, estrus, lameness, etc. The objective of this study was to investigate, in high producing cows, the modifications occurred during RH cases. Overall, 50 RH cases were identified in 40 lactating Holstein cows across 7 different studies. Identified major causes were housing changes (TS, from freestall to tie stall, n = 15) and primary (PY, no apparent other causes, n = 28). Minor causes were lameness (n = 1), mastitis (n = 3), veterinary practices (n = 4) and estrus (n = 2). From each episode, we considered 3 d before (−3, −2, −1) the event, the first and the last day of RH (0) and the first 3 d of recovery (+1, +2, +3). Average RH length was 4.3 ± 0.9 d. Reticular pH (r-pH) was recorded by boluses and RT by collars. All data were statistically analyzed using JMP (v14.3) with a factorial arrangement for time and RH causes (TS and PY) using mixed model procedure, with day as repeated measure. The results showed a dramatic drop in RT (−270 min, from −3 to 0, $P < 0.01$) with more descendent slope in TS. We confirmed 200 min of RT as threshold of RH. During RH, DMI and milk yield decreased (−3 and –4 kg, from −3 to 0, $P < 0.01$) with PY as less productive group (−5 kg/d during the entire study). This fact showed how cow subjected to PY-RH were less productive also before the RH cases. In PY group, daily r-pH increased in d −1 and 0 (+0.06, $P < 0.05$), as daily r-pH st.dev. increased in d0 and a spike of pH below 5.5 was recorded (+40min and +6AUC, $P < 0.05$). During the recovery period, high variability in all parameters was recorded, depending on the different outcome of the cows. However, DMI and milk yield level was restored faster and completely in TS. In conclusion, PY-RH cases are characterized by anomalies in ruminal fermentation that can be better detected by measuring variation in daily pH, and not just the 5.5 threshold, as reported in literature. This fact is mainly due to the reticular localization of boluses and the peculiarity conditions at that level.

Key Words: rumination time, rumen hypomotility, pH

412 Evaluating the relationship between in vitro and in situ starch degradation rates. C. B. Gleason*[1], L. M. Beckett[2], B. R. dos Reis[1], M. B. Hall[3], and R. R. White[1], [1]*Virginia Tech*, *Blacksburg, VA*, [2]*Purdue University*, *West Lafayette, IN*,

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Determining starch degradability is important because of its implicit relation to gut health, energy availability, feed intake, and animal productivity. In vitro assays for protein and fiber degradability have been useful tools for ration formulation; however, analogous assessments of starch degradability have limited update. Our objective was to test how an in vitro starch degradation assay compared with the traditional in situ method. Samples of 28 livestock feeds were obtained from 3 feed mills and subjected to in vitro and in situ starch degradation analyses. Seven ruminally cannulated wethers (Suffolk, Dorset or Suffolk x Dorset) were used for the in situ procedure. In vitro starch degradation was assessed using the acetate buffer technique with modified enzymatic incubation times: amylase (AML) 5 min and amyloglucosidase (AGS) 10 min, AML 10 min and AGS 20 min, AML 15 min and AGS 30 min, AML 30 min and AGS 60 min, AML 45 min and AGS 90 min, and AML 60 min and AGS 180 min. R statistical software was used for all analyses. In situ rates were determined using the Ørskov method and in vitro rates calculated assuming exponential decay. Relationships were analyzed as a linear mixed-effects model with in situ degradation rate as the response variable and in vitro degradation rate, percent starch, percent crude protein, percent neutral detergent fiber, percent fat, and feed type as fixed effects. Interactions between in vitro degradation rate and the nutrient percentages were also included. Feed mill was included as a random effect. In situ degradation rates ranged from 2.5 to 620.0%/h and in vitro degradation rates ranged from 12.0 to 610.0%/h. A relationship ($P = 0.031$) between in vitro and in situ methods was observed but an interaction ($P = 0.042$) between in vitro degradation rate and neutral detergent fiber content was also significant, suggesting the consistencies among methods are influenced by fiber content. Additional work is needed to confirm the usefulness of this in vitro screening technique.

Key Words: in situ incubation, in vitro assay, ruminal starch degradability

413 A network analysis of continuous culture fermentation data.

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Network analysis is a valuable tool enabling holistic exploration of all possible relationships among variables, including the potential strength of those relationships, and is an ideal tool to evaluate relationships within complex, interacting environments like the rumen. The objective of this study was to leverage network analysis to evaluate relationships among degradable nutrient supplies, nitrogen dynamics, and volatile fatty acid (VFA) concentrations from 3 continuous culture fermentation experiments. Data were obtained from Latin Square continuous culture fermentation experiments conducted at The Ohio State University between 2012 and 2016. Eighteen nodes of interest were identified and included VFA concentrations; non-NH_3; non-microbial N; non-NH_3 N; bacterial N; NH_3 and CH_4 concentration; degradability of NDF and OM; fluid passage rate; and total protozoa concentration. Because of the hierarchical nature of the raw experimental data, variables of interest were initially regressed in univariate models with random effects representing experiment, fermenter, and period to determine significant network edges. The resulting network was mapped using the network package of R Statistical Software. Fermentation variables are highly interrelated, and all relationships presented are significant ($P < 0.05$). Concentration of major VFA was correlated with NH3 concentration, total protozoa concentration, CH_4 concentration and non-NH_3 N concentration. Concentration of minor VFA was associated with major VFA concentrations and fluid passage rate. Degradability of NDF and OM was related to total protozoa count and major VFA concentrations. Bacterial N flow was correlated with major VFA concentrations, NDF digestibility, CH_4 concentration, OM degradability, NH_3 concentration and non-NH_3 N flow. Protozoa concentrations were associated with NH_3 concentration, non-NH_3 N concentration, CH_4 production and bacterial N flow. The degree of redundancy in this network highlights the need to evaluate the rumen fermentation system as an ecosystem, rather than a set of single interventions and responses.

Key Words: methane, rumen protozoa, volatile fatty acids

Ruminant Nutrition: Carbohydrates and Lipids
Roundtable: Lipid Metabolism – A DC36 Recap

414 Recap of 36th Discover Conference on Lipids in Dairy Nutrition. K. Harvatine*, *Penn State University*, *University Park, PA*.

The 36th Discover Conference (DC36) titled “Lipids in Dairy Nutrition: From Feed to Milk Fat” was held in May 2019. The conference led off with a view of world markets and future supply and demand for fats and oils. The first session focused on lipid supply and covered the fundamentals of lipids in feeds to current methodology for analysis. This was followed by a discussion of managing rumen available fatty acid level including potential for modeling. Subsequent sessions were dedicated to understanding intestinal fatty acid digestibility both from meta-analysis and experimental approaches and bioactivity of fatty acids with specific focus on the mammary gland, adipose tissue, reproductive function, and nutrient partitioning. The conference was wrapped up with a discussion of aspects of milk fat important to dairy food processing and MIR analysis of milk fat. The current presentation will hit the major points covered at the conference and highlight key potentials identified and areas requiring further research.

Key Words: milk fat, lipids, DC36

Roundtable: Online Teaching: So What Now?

421 Putting theory into practice: Teaching strategies to increase student interest and engagement in introductory animal science courses. E. L. Karcher*, *Purdue University, West Lafayette, IN.*

Each year it is estimated that 58,000 new job openings are available for graduates with a bachelor's or more advanced degree in food, agriculture, renewable resources, or the environment. However, only 61% of these jobs are filled with graduates in these areas of expertise. One possible way to close this gap is to focus on classroom instructional strategies that can greatly influence student curiosity and interest in a subject. In introductory animal science courses, the majority of students have little or no previous knowledge or experience in agriculture. This provides an opportunity to increase interest and curiosity in animal production early in a student's undergraduate program. Specific classroom interventions, such as active learning strategies, create learning environments to foster student feelings of interest and curiosity. Implementation of active learning through social, authentic, problem-based activities develops student interest and motivation by supporting needs for autonomy and relatedness. Hands-on, problem-based activities may be more interesting and motivating to students compared with traditional lecture strategies. Additionally, first-year students in animal sciences self-report high levels of curiosity in animal science. The goal should be to continue stimulating this curiosity throughout the course and undergraduate program. Case studies and laboratory stations, designed with interactive and group-based instructional methods, have shown great benefit to sustaining curiosity in first-year animal science students. Classroom interventions targeting introductory courses have the potential to increase career awareness by stimulating student interest and curiosity in the subject. This integration may assist in closing the growing employment gap in the agricultural sciences.

Key Words: active learning, motivation, undergraduates

422 Uses of technology to increase interest and learning. M. A. Wattiaux*, *University of Wisconsin-Madison, Madison, WI.*

Learning theories have made it clear that interest and motivation are essential to the learning process. Instructional technology is often instructor-centered (what can technology help me accomplished in my class?), but it can have both beneficial or detrimental impacts on students' engagement with each other and with course content. Our objective was to illustrate the use of technology to enhance the quality and effectiveness of in-class activities in small enrollment, discussion-based courses with emphasis on creating a student-centered learning community. In this study, data were collected via a mid-semester survey in a senior level ruminant nutrition course taught between 2012 and 2017 in which readings and quizzes were completed on-line prior to class, followed by in-class discussion of the reading material. Students quantified on a scale of 1 (not at all) to 10 (a great deal) the extent to which each of the following components of the class help their learning: (a) the readings, (b) the web-based quizzes, (c) the in-class discussion, and (d) the explanations given on the chalkboard. Contribution of each class component to the teaching and learning environment of the class was calculated for each student as a deviation from self-reported "overall level of learning"; in the class, which was quantified elsewhere in the survey using the aforementioned scale. Overall level of learning was (mean ± SE; n = 153) 7.3 ± 0.13 and average deviation for (a) reading, (b) online quizzes, (c) in-class discussion and (d) use of the chalkboard was −0.82 ± 0.16, −0.27 ± 0.14, 0.63 ± 0.14, and 0.64 ± 0.17, respectively. Students perceived more learning with in-class activities (c and d) than activities (a and b) completed online prior to class. Although, students did not feel confident that reading contributed "positively" to the learning environment of the class, the on-line quizzes provided them with a certain degree of self-efficacy. These data suggested also that the use of a chalkboard to explain, or more generally to capture and share elements of a discussion in writing, may be a powerful complement to the thinking, speaking, and listening that takes place during in-class discussions.

Key Words: pedagogy, teaching and learning.

423 Motivation and interest in online courses. M. D. Stern*, A. E. Neu, and K. L. Martinson, *University of Minnesota, Saint Paul, MN.*

Popularity of online learning is increasing so rapidly that it is difficult to estimate growth. A survey published in 2016 showed that 31% of higher education students take at least one online course during their collegiate career and the rate of growth for online enrollment exceeds that of over-all higher education student enrollment. Because of demographic changes of animal science students, an introductory Companion Animal Nutrition and Care course was developed to meet the demand of urban and pre-veterinary students. To make this course more accessible campus-wide, an online section was offered as an elective to all majors. Because of the interest in equine species, Horse Management is another popular introductory animal science course offered in-person and online at the University of Minnesota (UMN). Data were collected using these two courses for two consecutive academic years to assess learning gains and satisfaction for the two delivery methods. Learning gains were assessed by comparing pre vs post-test scores. Learning gains (from 12 to 41%) occurred in both courses and delivery methods, with in-person students achieving greater gains in three of the four course years ($P \leq 0.01$). Satisfaction was evaluated using questions administered through the UMN's Student Rating of Teaching (SRT). Satisfaction was high among both courses and delivery methods (>4.8 on a 6.0 scale), although in-person students reported higher ratings compared with online students in some instances ($P \leq 0.01$). When evaluating undergraduate, introductory-level online and in-person courses, students utilizing both delivery methods experienced learning gains and were satisfied. Based on these results, online courses can be successfully used to teach undergraduate students introductory-level animal science courses. Another approach to using online tools to interest students in upper level animal science courses such as Ruminant Nutrition, is to restructure and offer a blended-course (in-person and online). Because of decreases in dairy/beef production students, the course was also revamped to include exotic ruminants. These modifications to the course doubled student numbers.

Key Words: teaching, online, learning

ADSA-SAD Original Research Poster Competition

M1 Foaming and baking properties of spray-dried ingredient from whey protein and pectin complexes. J. Xu* and B. Vardhanabhuti, *University of Missouri*, *Columbia, MO*.

Whey proteins are the major food ingredients; however, they are underutilized in food foams compared with egg white protein (EWP). Previous work showed that heated soluble complexes (CPX) formed by heating whey protein isolate and pectin exhibited improved foaming properties. For industrial applications, the CPX need to be spray-dried. The objective of this study was to investigate the foaming and baking properties of spray-dried CPX powder (SP-CPX). SP-CPX solutions (1–5% protein) were prepared, and their foaming properties were compared with EWP. Foams were generated using a KitchenAid mixer. Foaming properties were determined by measuring the overrun and drainage 1/5 life. Finally, mixed SP-CPX and whey protein concentrate (WPC) solution (15% protein) was prepared at 12:88 protein ratio. The solution was used in angel food cake formulation. Baking properties were determined by measuring cake volume and density. As comparison, cakes were also made using EWP and WPC. All experiments were performed in duplicate. Analysis of variance and post-hoc Tukey's HSD were used to determine the statistical difference ($P < 0.05$). Results showed that, at 1%, SP-CPX could form stable foams with 761% overrun and 11 min drainage time while EWP foams were not stable. Increasing to 2% protein led to a significant increase ($P < 0.05$) in foam overrun for SP-CPX and the formation of stable EWP foams. Further increase in protein concentration did not affect the overrun ($P > 0.05$), and there was no significant difference between SP-CPX and EWP. Increasing protein concentration led to increased drainage time; however, SP-CPX foams were significantly more stable than EWP. At 4 and 5% protein, SP-CPX foams did not drain after 150 min. Foaming properties of SP-CPX were proven in angel food cakes. Without SP-CPX, cakes made with WPC had lower volume (267 ± 3 mL) and higher density (0.84 ± 0.01 g/mL). Partially replacing WPC with SP-CPX led to increased volume (434 ± 0 mL) and decreased density (0.53 ± 0.00 g/mL). Cakes made with mixed WPC and SP-CPX were similar to those made with EWP. These results indicate that SP-CPX powder has excellent foaming properties and can be used to improve the baking properties of WPC.

Key Words: complexes, whey protein

M2 Effects of starch and fat concentrations in starter grain on Jersey calf performance. E. Sgambati* and M. Eastridge, *The Ohio State University*, *Columbus, OH*.

The development of replacement heifers is important for maintaining herd size and financial efficiency of dairy farms. We investigated the nutritional needs of Jersey calves, focusing on meeting energy requirements by altering starch and fat concentrations in calf starters. Thirty-six female Jersey calves were grouped by BW and birth date and randomly assigned among 3 calf starters: 35% starch and 2% fat (HST), 20% starch and 2% fat (LST), and 35% starch and 4% fat (HST-F). The fat supplement for HST-F consisted of 20% coconut oil, 45% lard, 15% flaxseed oil, and 20% soybean oil. All of the ingredients were pelleted except the steam-flaked corn and molasses. Calves were fed 4 L of colostrum at birth. All calves were fed the same milk replacer at 4.4 L/d during week 1 of age, 5.2 L/d during wk 2 to 7, and 2.6 L/d during wk 8 before weaning. Intake as measured daily and wither heights and BW were measured weekly. One week after weaning, fecal and feed samples were collected daily for 3 d with the fecal collections representing 24 h/d. Using acid insoluble ash, apparent digestibility of dry matter (DM), neutral detergent fiber (NDF), and crude protein (CP) were determined. Neither starter (730, 693, and 716 g/d, respectively for HST, LST, and HST-F) nor total DM intakes (1259, 1209, and 1245 g/d, respectively) differed among treatments, and there were no treatment by week interactions. Average daily gains from birth through wk 10 of age were similar among groups (0.586, 0.534, and 0.550 kg/d, respectively), thus BW and wither heights were similar across weeks. Digestibilities of DM (73.4, 66.0, and 70.2%, respectively), NDF (41.6, 50.2, and 41.4%, respectively), and CP (67.6, 65.1, and 62.6%, respectively) were similar among treatments. Fecal scoring was evaluated on a 1–6 scale, with 6 indicating hard, dry feces and 1 indicating runny, liquid feces. For the first 4 wk of age, there were no differences among treatments for average fecal scores and days with fecal scores of 1 to 4. Starch and fat concentrations in the starters appeared to not affect calf performance which can provide flexibility in formulation of starters based on ingredient costs.

Key Words: calf, starch, Jersey

M3 Effects of intramammary infections on colostrum quality in Jersey cows. E. M. Hist*, N. R. Hardy, K. M. Enger, and B. D. Enger, *The Ohio State University, OARDC*, *Wooster, OH*.

A high-quality colostrum with sufficient antibody concentrations is essential for immune health in the newborn calf. Colostrum is highly variable between cows and among quarters within a cow. Intramammary infections (IMI) often occur during the time of colostrum formation; however, it is unknown if these infections ultimately affect colostrum quality and antibody concentrations. The objective of this study was to determine if antibody concentrations in colostrum from infected mammary glands differed from uninfected. Colostrum was collected from 90 cows across 4 Ohio dairy farms within 12 h of calving. Colostrum samples were aseptically collected from all 4 quarters of the cow by farm personnel and refrigerated at −4°C. Within 24 h of collection, Brix degrees were quantified using a refractometer and samples were cultured to determine infection status. Of the 353 quarters sampled, 88 quarters had an IMI. Antibody concentrations were quantified and compared within cow when a comparison was available among a pair of rear or fore quarters, one infected and one uninfected. This resulted in 8 primiparous quarter pairs and 10 multiparous pairs to be evaluated by ELISA. Statistical analyses were performed in SAS using the MIXED procedure with infection status and parity included as fixed effects; cow nested within farm was a random effect. Overall, Brix degrees were greater in primiparous cows than multiparous (27.2 vs 24.3 ± 1.2; $P < 0.05$). Infected quarters had lower Brix degrees than uninfected in multiparous cows (23.6 vs 25.1 ± 1.0; $P < 0.01$), but this was not observed in primiparous cows. Of the paired samples, concentrations of IgG2 tended to be lower in multiparous cows than primiparous cows (2.9 vs 4.5 ± 0.7mg/mL; $P = 0.11$). Concentrations of IgA, IgM, and IgG1 did not differ based on infection status or parity. Ultimately antibody concentrations were not highly influenced by infection status, but differences were seen between parity groups. These results indicate that variability in colostrum quality is more greatly influenced by factors other than infection status.

Key Words: antibody

M4 Effects of *Yucca schidigera* based additive on in vitro dry matter digestibility, efficiency of microbial production, and greenhouse gas emissions. X. L. Crumel*[1], D. P. Compart[2], and U. Y. Anele[1], [1]*North Carolina Agricultural and Technical State University*, *Greensboro, NC*, [2]*PMI*, *Arden Hills, MN*.

Two in vitro batch culture experiments were conducted to evaluate the effects of Saport Feed Additive (Saport, PMI, Arden Hills, MN), a *Yucca schidigera* based feed additive, on in vitro dry matter digestibility (IVDMD), short chain fatty acids, microbial mass, efficiency of microbial production and greenhouse gas emissions. In experiment 1, 7 concentrations of Saport were evaluated using alfalfa hay and a concentrate diet to determine the optimum dose to use in experiment 2. The concentrations were 0 (control), 0.5, 1, 2, 4, 6, 8 g/head/day. Based on the results, 2 g/head/day was selected for experiment 2. For the second batch culture study, 4 dairy diets (2 corn silages and 2 TMR; collected from different farms in Guilford county, NC) were used as substrates and incubated for 3, 6 and 24 h using rumen fluid from 2 ruminally cannulated dairy cows. Treatments were 0 (control) and 2 g/head/day of Saport. Inclusion of Saport had no effect ($P = 0.7869$) on both apparently and truly degraded DM. Additionally, Saport had no effect ($P > 0.05$) on short chain fatty acids, microbial mass and efficiency of microbial production. Methane production was reduced by 22.7% with Saport inclusion and this was consistent among the 4 diets. Similarly, lower ($P = 0.0132$; 18%) carbon dioxide concentration was observed in Saport treatment. Ammonia and hydrogen sulfite concentrations were similar ($P > 0.05$) for both treatments. However, Saport inclusion at 2 g/head/day did reduce ammonia and hydrogen sulfite concentrations by 13 and 17%, respectively. Regardless of diet type, inclusion of Saport at 2 g/head/day reduced methane and carbon dioxide concentrations. The reduction in greenhouse gas emissions did not result in any increase in either short chain fatty acids, microbial mass or efficiency of microbial production.

Key Words: batch culture, greenhouse gases, *Yucca schidigera*

M5 Effect of chemical and biological preservatives on the dry matter loss, nutritional composition, microbial counts, and heating of aerobically exposed wet brewer's grain silage. R. Hollandsworth*[1], M. Killerby[1], S. T. R. Almeida[2], Z. X. Ma[3], A. Y. Leon-Tinoco[1], B. C. Guimaraes[2], G. M. Oppong[1], and J. J. Romero[1], [1]*University of Maine*, *Orono, ME*, [2]*University of Lavras*, *Lavras, Brazil*, [3]*University of Florida*, *Gainesville, FL*.

Our objective is to identify the most effective preservatives of wet brewer's grain (WBG) silage, especially during the feedout phase. Treatments (TRT) were sodium lignosulfonate at 1% (NaL1) and 2% (NaL2; wt/wt of fresh WBG), propionic acid (PRP; 0.5% wt/wt of fresh WBG), a combination inoculant (INO; *Lactococcus lacti*s and *Lactobacillus buchneri* each at 4.9 log cfu/fresh WBG g), and untreated (CON). Five batches of unroasted WBG were collected from the same source right after lautering and cooled to room temperature for 1 d before treatments were randomly applied to each batch. Treated WBG were packed into 8.8 L mini-silos and stored for 60 d at 21°C. At opening, PRP had a lower DM loss (5.06) and a markedly better preservation of sugars (25.0) relative to all other TRT (~10.5 ± 1.26% and ~13.6 ± 1.36% of DM). Afterward, WBG silage was aerobically exposed for 10 d. Data were analyzed as a randomized complete block design (5 blocks) and differences were declared at $P \leq 0.05$. NaL2 had the highest DM (23.6) and pH (4.96) and lowest NDF (38.3) relative to the other TRT (~22.9 ± 0.158%, ~4.42 ± 0.126, and ~50.4 ± 1.03). PRP DM loss during aerobic exposure (11.7) was no different than CON (13.3) but both were lower than the other TRT (~18.2 ± 1.19%). CON (3.24) had the lowest sugar concentration relative to PRP and NaL2 (~7.59) and INO (6.21 ± 0.778% of DM). Moreover, PRP had the lowest ash concentration (3.92) relative to CON (4.25) and NaL1 and 2 (~5.89 ± 0.104% of DM). PRP had a higher yeast count (8.23) relative to INO (6.69), but no different than CON (7.41 ± 0.433 log cfu/g). Heating degree days across TRT (68.5) were similar to CON (62.2), except for NaL1 (86.0 ± 6.42 ?-d). Furthermore, NaL1 and 2 had higher maximum temperatures (~38.7) relative to the other TRT (~36.5 ± 0.82?). Considering that at silo opening PRP had preserved much more nutrients (especially sugars) than CON and thus was more susceptible to aerobic spoilage, its DM loss during aerobic exposure was no different than CON and it still preserved more sugars.

Key Words: silage, lignosulfonates, wet brewer's grain

M6 Assessment of the effects of intrauterine dextrose infusion on clinical cure rate, daily milk yield, and daily rumination in postpartum dairy cows diagnosed with clinical metritis. J. Hamilton*[1], A. A. Barragan[1], E. Hovingh[1], L. Byler[1], M. Martinez[1], S. Bas[2], J. Zug[3], and S. Haan[3], [1]*Department of Veterinary and Biomedical Sciences, Penn State University*, *University Park, PA*, [2]*Phytobiotics Futterzusatzstoffe GmbH Bvd*, *Villa Maria, Córdoba, Argentina*, [3]*Zugstead Farm*, *Mifflintown, PA*.

The objective of this study was to assess the effects of intrauterine dextrose (50%) infusion on clinical cure rate, daily milk yield and daily rumination time in dairy cows diagnosed with clinical metritis (CM). Cows (n = 351) from a dairy farm located in Pennsylvania were screened at 7 ± 3 DIM using a Metricheck device to assess vaginal discharge. Cows that presented a fetid red-brownish watery vaginal discharge (n = 53) were classified as CM cows, blocked by parity and randomly assigned to 1 of 2 groups: 1) CONV (n = 27): 2 injectable (SC) administrations of ceftiofur (6.6 mg/kg; Excede, Zoetis Inc.) 72 h apart; and 2) DEX (n = 26): 3 intrauterine infusions of dextrose (50%; 1 L/cow) 24 h apart. Furthermore, cows that presented a normal vaginal discharge at 7 ± 3 DIM (i.e., dense clear discharge; healthy group; HLT; n = 27) were randomly selected and matched by parity to CONV and DEX cows. Cows were re-screened at 14 ± 3 DIM and 21 ± 3 DIM to assess clinical cure rate. Daily milk yield and rumination time data for the first 150 DIM were collected from on-farm computer records (SCR Dairy, Netanya, Israel). The data were analyzed using the MIXED and GLIMMIX procedures of SAS as a randomized complete block design. There was no difference in clinical cure rate at 14 ± 3 DIM (CONV = 50.27 ± 12.49%; DEX = 45.9 ± 12.37%) and 21 ± 3 DIM (CONV = 88.05 ± 6.59%; DEX = 88.51 ± 6.35%) between CONV and DEX groups. Overall, cows in the CONV and HLT groups tended to produce 3.41 kg/d and 3.71 kg/d more milk, respectively, compared with cows in the DEX group during the first 150 DIM (CONV = 43.46 ± 1.22 kg/d; DEX = 40.04 ± 1.18 kg/d; HLT = 43.75 ± 1.20 kg/d). There was no difference in daily rumination time during the first 150 DIM between study groups (CONV = 564.29 ± 8.21 min/d; DEX = 542.29 ± 8.09 min/d; HLT = 553.94 ± 7.98 min/d). The results from this study suggest that although intrauterine infusion of dextrose may achieve similar clinical cure rates as a conventional antibiotic treatment, the treatment may not be as effective at restoring milk production in CM cows.

Key Words: clinical metritis, intrauterine dextrose, milk yield

M137 On-farm assessment of insulin sensitivity from glucose responses to insulin infusion in neonatal dairy calves. N. P. Uzee*[1], C. C. Williams[2], E. L. Oberhaus[2], S. J. Blair[2], and B. S. Whitley[1], [1]*Louisiana State University, Baton Rouge, LA*, [2]*LSU AgCenter, Baton Rouge, LA*.

Six male Holstein calves were used to assess glucose responses to insulin to develop an on-farm method of measuring insulin sensitivity with 4 blood samples. Calves were housed in individual hutches and fed milk replacer twice daily according to manufacturer recommendations. Milk replacer was reduced to one feeding daily on d 42, with weaning on d 49. Calf starter and water were offered free choice beginning on d 3. At 3, 6 and 9 weeks of age, insulin tolerance tests were conducted at 0700 h. Calves were not fed the AM feeding or allowed access to feed for the duration of the test. Insulin (7.5 mU/kg BW, 15 mU/kg BW, 30 mU/kg BW, 45 mU/kg BW, or 60 mU/kg BW) was infused through a jugular catheter (14 gauge, 3.5 in; MILA International, Inc.; Erlanger, KY) at time 0. Insulin concentrations were selected based on calf age, with calves at 3 wk receiving 7.5, 15, and 30 mU/kg and calves at 6 and 9 wk receiving the higher doses. Blood was collected at −10 and 0 min pre-insulin infusion and 10, 20, 30, 40, 50, 60, and 70 min post- insulin infusion and immediately tested for glucose concentrations using an Abbot Precision Xtra meter. The percentage decline in glucose concentrations at 40 and 60 min was calculated and plotted against the natural log (ln) of the insulin dose for each calf. Linear regression analysis was used to calculate the regression equation for each calf, and the ln of the dose of insulin resulting in a 50% decline in glucose concentration [ln(ED50)] was estimated from that equation. The ED50 was calculated by taking the antilog of ln(ED50). These values were used to determine the insulin dose that best predicts insulin sensitivity. Results indicated that mean insulin concentrations of 13, 33, and 36 mU/kg BW at 3, 6, and 9 weeks, respectively, were determined to result in a 50% decline in blood glucose post infusion using only 4 blood samples (−10, 0, 40, and 60 min post insulin). This method for assessing insulin sensitivity with fewer blood samples and no laboratory analyses will benefit researchers in quickly generating research data in dairy calf projects.

Key Words: insulin sensitivity, dairy calves, on-farm assessment

M7 Application of micro-nano-bubbles to improve the processability of milk protein concentrates. K. Sajith Babu* and J. Amamcharla, *Kansas State University*, *Manhattan, KS*.

Micro-nano-bubbles (MNB) have unique properties and offer potential applications in various fields. The first objective of this study was to confirm the MNB injection method is capable of producing sufficient MNBs. The bubble particle size and concentration in deionized (DI) water and MNB treated DI water was measured using a nanoparticle tracking system. MNB treated DI water had a mean particle size of 249.8 ± 115.8 nm and a bubble concentration of 3.76×10^8 particles/mL. In other words, the concentration of MNB treated DI water was significantly higher compared with the DI water (>300 million nano-bubbles/mL). The second objective of this study was to evaluate the effectiveness of MNB treatment to improve the rheological and functional properties of milk protein concentrate (MPC). Reconstituted MPC (20%, wt/wt) passed through a pump was considered as control (C-MPC), and MPC passed through the MNB system was considered as MNB treated (MNB-MPC). The rheological behavior and microstructure of C-MPC and MNB-MPC were evaluated. The viscosity of MNB-MPC at shear rate of 100 s^{-1} significantly decreased ($P < 0.05$) from 0.165 to 0.03 Pa.s (C-MPC). The C-MPC and MNB-MPC were spray dried in a lab-scale spray dryer and the rehydration characteristics, bulk and shear flow properties, and morphology were evaluated. Focused beam reflectance measurement of the MNB-MPC powders showed higher counts of fine particles during dissolution, which indicated MNB-MPC powders were more soluble than C-MPC powders. Conditioned bulk density and flow function coefficient were significantly higher ($P < 0.05$) for the MNB-MPC powders. MNB-MPC powders were more free-flowing as observed from significantly lower ($P < 0.05$) basic flow energy values compared with the C-MPC powders. MNB-MPC powders exhibited less cohesiveness and better flow characteristics than C-MPC powders. This study, therefore, confirms the potential of using MNB treatment for more efficient drying and improved functionality of MPC powders.

Key Words: milk protein concentrate, micro-nano-bubbles, rheology

M8 Tracking microbiome shifts throughout cheddar cheese production. J. Choi[1], B. Rackerby*[1], S. Lee[1], R. Frojen[1], L. Goddik[1], S. Ha[2], and S. Park[1], [1]*Department of Food Science and Technology, Oregon State University*, *Corvallis, OR*, [2]*Department of Food Science and Technology, Chung-Ang University*, *Ansung, Geonggi-Do, South Korea*.

It is essential for the dairy industry to understand the microbial community structure of cheese as manufacturing practices influence the microbiome which in turn drives safety and sensory characteristics, thereby affecting the quality of the cheese. While some organisms impart desirable characteristics, others are associated with a reduction in quality. To characterize the shifts in microbial community structure throughout the cheddar cheese making process, 3 batches of cheddar cheese were produced at the Oregon State University Creamery on April 26, June 6, and June 26, 2018. Samples were collected from 36 time points throughout processing and separated into 3 stages: pre-inoculation, production, and aging (6 mo). Environmental samples were collected from the facility to identify a source of non-starter lactic acid bacteria (NSLAB), contaminants of cheese which often impart negative characteristics. DNA was isolated from all samples and sequenced at the 16S rRNA V4 region using an Illumina MiSeq to analyze differences in microbial populations. All cheese samples were predominated by *Streptococcus* and *Lactococcus,* both of which were present in the starter lactic acid bacteria (SLAB). Raw and pasteurized milks showed high levels of α diversity before the addition of SLAB. Upon SLAB inoculation, microbial richness decreased significantly and microbial compositions shifted. NSLAB appeared 15 weeks after aging and were determined to be an unidentified group of *Lactobacillaceae*. Beta diversity analysis revealed that community structure changed sequentially throughout production, and that the April cheese differed from the 2 June produced cheeses, indicating that microbiota differ across both production stage and batch. Functionally, post-inoculation samples from production and ripening clustered together, sharing similar functional properties that differed significantly from pre-inoculation samples. Addition of SLAB reduced the microbial richness and shifted the microbial communities to be more similar both compositionally and functionally. A source of NSLAB was not determined, however growth was present in 39 of 72 samples.

Key Words: cheese, microbiome, high-throughput sequencing

M9 Characterization of adhesion property between *Lactobacillus reuteri* and milk phospholipids, and the gene expression of binding-promoting proteins. L. Zhang*, I. García-Cano, and R. Jiménez-Flores, *Department of Food Science and Technology, The Ohio State University*, *Columbus, OH*.

Due to the beneficial effects of milk phospholipids (MPLs) and lactic acid bacteria (LAB), the application of using them as a combination is getting scientists' attention and has promoted research on analyzing their nutritional and functional properties. Recently, a strain-specific adhesion property between MPLs and LAB, especially *Lactobacillus reuteri*, was observed. This study aims to quantify such adhesion property and investigate its correlation to gene expression of 3 binding-promoting proteins: mucus adhesion-promoting protein (mapA), cell and mucus-binding protein A (cmbA) and collagen-binding protein (cnb). Out of 122 LAB cultivated in MPLs supplemented MRS medium, 27% demonstrated an adhesion property, 28% showed a "semi-adhesion" property, while the rest separated from the MPLs layer after incubation. It also indicated that the adhesion property was strain-specific −38% of LAB demonstrating the adhesion property with MPLs was *L. reuteri*. Six *L. reuteri* strains and 6 strains from other species were selected to quantify the adhesion property using a modified sucrose density gradient centrifugation method. Three binding-promoting proteins presented in *L. reuteri* were selected, and the gene expression of them in selected bacteria were analyzed using RT-qPCR in triplicates. The change of gene expression was calculated with the paired *T*-test. The criterion for significance was set at $P < 0.05$. Pearson correlation coefficient was used to evaluate the correlation between gene expression level and adhesion property. The result indicated that the gene expression level of all 3 proteins were not significantly changed by the addition of MPLs into MRS medium. However, in *L. reuteri*, the gene expression level of cmbA showed a significant correlation with the adhesion property. This study suggests that the addition of MPLs may not likely to affect the mucus adhesion property of LAB. Meanwhile, the combination of MPLs and *L. reuteri* with a high cmbA expression level might contribute to the delivery of MPLs into gut microbiota upon consumption.

Key Words: milk phospholipids, lactic acid bacteria, adhesion property

M10 Effect of soluble casein isolate on the functionality and mechanical properties of milk protein concentrate (MPC80) during storage. Y. Zhu*[1], M. S. Molitor[2], S. Ikeda[1], and J. A. Lucey[2], [1]*Department of Food Science, University of Wisconsin-Madison*, *Madison, WI*, [2]*Center for Dairy Research, University of Wisconsin-Madison*, *Madison, WI*.

MPC80 is a high-protein milk powder widely used in the food industry as a functional ingredient. A problem associated with MPC80 is its poor solubility, which can further deteriorate during storage at high temperature and humidity. A new approach to improve MPC solubility was investigated. The Center for Dairy Research (CDR) has developed a technology to produce soluble casein isolate (SCI), which is very similar to sodium caseinate but made via a membrane filtration method. Various levels (0, 1, 5, 10%) of soluble casein isolate (SCI), manufactured by CDR, was added to the UF retentate before spray drying and the MPC powders were stored at 30°C for up to 60 d after manufacture. The above processes were replicated twice, using different lots of skim milk. The results showed that addition of SCI did not change the particle size distribution or disrupt the casein micelle structure. Adding 5% or more SCI (in final dry matter) significantly improved MPC solubility and foam capacity on d 0 ($P < 0.05$), but the foam capacity was not significantly different after 60 d storage ($P > 0.05$). Atomic force microscopy (AFM) operated in Peak Force QNM mode was used to investigate the nanomechanical properties of powder particle surfaces. The powder particle surface topography was hardly impacted by storage under high temperature, however, storage under high temperature led to an increase in powder stiffness for all 4 samples and the elastic modulus of the MPC samples was negatively correlated with solubility ($r = -0.86$, $P < 0.001$). Addition of SCI, at levels 5–10% in final dry matter, could have increased the amount of non-micellar casein on powder surface and possibly slow down the case hardening of MPC powder particles during high temperature storage, which might have contributed to the improvement in solubility and functionality.

Key Words: milk protein concentrate, soluble casein isolate, atomic force microscopy Peak-force QNM

M11 The effect of micellar casein concentrate purity on sulfur/eggy flavor in UHT milk protein-based beverages.

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Micellar casein concentrate (MCC) is made by removing serum proteins (SP) from skim milk by microfiltration (MF). Milk SP denature during thermal processing and are the source of volatile sulfur/eggy off-flavors (H_2S and C_2S). Our objective was to determine the amount of milk derived whey protein (MDWP) removal (i.e., SP) required to eliminate sulfur/eggy flavor in milk protein beverages at 15 g protein / 240 mL serving. MCC with 95% MDWP removal was produced from skim milk (50°C) using a 3X, 3-stage ceramic MF process using 0.1 µm pore size graded permeability membrane. Model beverages with carrageenan, cellulose gum, and dipotassium phosphate were created by adding MDWP back into the MCC to simulate 4 levels of MDWP removal: 95.2%, 91.0%, 83.2%, and 69.2% which correspond to MCC purities (i.e., casein % of true protein) of 93.8, 93.2, 92.2 90.2%, respectively. Beverages were ultrapasteurized (UHT) by direct steam injection (140°C for 2.3 s), bottled and cooled (4°C). Residual SP and MCC purity were measured by Kjeldahl before UHT. Beverages were profiled by descriptive analysis and analyzed by gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) for sulfur volatiles (H_2S, C_2S). The H_2S concentrations were 9.3^d, 21.9^c, 35.4^b, 58.6^a ppb and C_2S were 33.3^d, 58.5^c, 100.7^b, 148.8^a ppb ($P < 0.05$) for beverages corresponding to 95.2, 91.0, 83.2, and 69.2% MDWP removal, respectively. The residual MDWP concentrations increased (0.40^d, 0.44^c, 0.51^b, and 0.63^a g/100 g beverage), as milk serum protein removal % decreased. Sensory perception of sulfur/eggy flavor intensity increased with increasing level of residual SP ($P < 0.05$). Concentrations of H_2S and C_2S were below documented threshold values, and sulfur/eggy flavors were not detected by the trained panel in the MCC with 95% serum protein removal. Serum protein removal from MCC must be greater than 91% to achieve a product with no sulfur off-flavors following thermal processing. To eliminate sulfur eggy flavor in a milk protein beverage at 15 g protein and 0.35 g fat per 240 mL serving, the residual SP concentration needs to be <0.96 g/240 mL beverage.

Key Words: micellar casein, purity, protein beverages

M12 Development of a novel milk protein concentrate containing whey proteins as fibrils.

G. Rathod* and J. Amamcharla, *Kansas State University*, *Manhattan, KS.*

Milk protein concentrate (MPC) is used to provide nutritional benefits, enhanced viscosity, and water holding in various food products. Altering the protein configuration and protein-protein interactions in MPC can provide a novel functionality in terms of gelling and viscosity. Hence, an attempt was made to modify MPC by converting whey proteins as fibrils. Whey protein fibrils are known to increase viscosity and water holding capacity compared with the native protein. The objective of this work was to evaluate the functionality of modified MPC by converting whey proteins as fibrils. For this purpose, a commercial native whey protein (nWP) and micellar casein concentrate (MCC) were procured from commercial sources. The nWP was dissolved in distilled water to make a 2% (wt/wt) solution and divided into 2 equal parts. The pH of the first part was adjusted to 2 and heated to 80°C for 20 h. Once the fibrillation was complete, the pH of the solution was adjusted to 6.7 and served as modified. The second part was served as control. Subsequently, the control and treated solutions were mixed with MCC to provide a final casein to whey protein ratio of 80:20 to resemble MPC with 10% protein. The control and modified MPC were analyzed in terms of viscosity, thioflavin T (Th T) value, particle size, and morphology. All the experiments were carried out in duplicate. Transmission electron microscopy images confirmed the presence of whey protein fibrils in the modified whey protein solution. However, the number of fibrils were visibly low after pH adjustment and MCC addition. The apparent viscosity at low shear rates was significantly ($P < 0.05$) higher for modified MPC compared with control MPC. Both the samples showed a shear thinning behavior with flow behavior index less than one. The consistency coefficient for control and modified MPC solution was found to be 0.0015 ± 0.0005 and 11.8696 ± 0.1368 Pa.S^n, respectively. The Th T fluorescence value of modified MPC (792 ± 185 AU) was higher than the control sample (632 ± 186AU). Overall, modified MPC containing whey proteins as fibrils showed promising differences when compared with the regular MPC.

Key Words: modified milk protein concentrate, viscosity

M13 Impact of palmitic acid and pH on ruminal NDF digestibility and fermentation in a continuous culture system. L. Padilla*, A. Sears, and F. Batistel, *Department of Animal, Dairy and Veterinary Sciences, Utah State University*, *Logan, UT.*

Non-rumen bacteria incorporate exogenous long-chain saturated fatty acids to change membrane fluidity under low pH conditions. We hypothesized that rumen bacteria use a similar mechanism, thus, providing saturated fatty acids in the diet could support bacterial metabolism and growth and consequently enhance fiber digestibility. The objective of this study was to evaluate the effects of dietary palmitic acid and pH on ruminal NDF digestibility and fermentation. The study was conducted as a 2 × 2 factorial treatment arrangement in a replicated 4 × 4 Latin square using continuous culture fermenters (n = 8). Treatments were a control diet without supplemental fatty acids or the control diet plus 1.5% of palmitic acid factorialized with normal pH (diurnally ranging from 6.4 to 7.0) or low pH (diurnally ranging from 6.0 to 6.4). The control diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate mixture that provided 5.8 g CP, 14 g NDF, 7.3 g starch, and 1 g fatty acids fed twice daily. The fatty acid treatment maintained the same nutrient input into the fermenters as the control except for fatty acids. Both buffer solutions (normal and low pH) were delivered continuously at rate of 10%/h. Data were analyzed using a mixed model including the fixed effect of pH, fatty acid, and its interaction, and the random effects of period and fermenter. Data are reported as least squares means with differences declared at $P \leq 0.05$. No interaction between fatty acids and pH were observed for the variables measured. Compared with control, palmitic acid increased NDF digestibility (45.2 vs. 39.34%, $P = 0.03$). The lower pH decreased NDF digestibility in 8.2 percentage units compared with normal pH (46.4 vs. 38.16%, $P < 0.01$). Furthermore, low pH decreased ammonia (7.30 vs. 5.64 mg/dL, $P = 0.01$) and total VFA concentration (168 vs. 138 mmol/d, $P = 0.02$) compared with normal pH; palmitic acid did not ($P > 0.10$) affect ammonia nor total VFA concentration. Our preliminary data indicate that rumen pH and palmitic acid independently affect NDF digestibility and rumen fermentation. Palmitic acid supplementation increased ruminal fiber digestibility under low and normal pH conditions.

Key Words: fatty acids, rumen, pH

M14 Effects of fatty acid supplementation to prepartum dairy cows on immunity in dams and their offspring. A. Schroeder* and M. Eastridge, *The Ohio State University*, *Columbus, OH.*

The objectives of this study were to investigate the effects of fatty acid supplementation differing in ω-6:ω-3 to prepartum dairy cows on colostrum yield and quality and immunity of the dam and calf. The focus is on improving passive immunity and long-term health measures in calves. 53 nonlactating pregnant Holstein cows were allocated to blocked and randomized amound 3 feeding groups considering parity and date of expected calving. All cows were fed ad libitum a TMR formulated at 14% CP and a DCAD of −7.3 mEq/100 g. The TMR consisted of corn silage (42.6%), grass haylage (28.4%), concentrate mix (27.3%), and top-dressed with 1 of 3 treatments consisting of 50% corn and 50% of the fat supplements (1.7%; ~0.227 kg/d): R4 (n = 18) with 23 g/d Prequel and 91 g/d Strata (Virtus Nutrition LLC, Corcoran, CA; ω-6:ω-3 (R) = 4); R6 (n = 18) with 64 g/d Prequel and 50 g/d Strata; and R8 (n = 17) with 91 g/d Prequel and 23 g/d Strata. Treatments were initiated at 21 d before expected parturition, at which time the cows were moved from group housing to individual maternity stalls. Feed offered and refused from individual cows were used to determine daily DMI; BW and body condition score (BCS) were determined at the beginning of the trial and weekly until calving. Blood was collected from the jugular vein when calving was imminent. Fresh animals were milked within 2 h of calving; 4L of fresh colostrum from the dam was bottle fed to each calf, and samples of colostrum and blood from the calf were collected at hour 0 and blood again at 48 h. The mixed model procedure of SAS was used for data analysis with block as the random variable. Data with $P < 0.05$ was regarded as significant and $P < 0.15$ a trend. DMI tended ($P = 0.11$) to be higher for R4 than R6 and R8 at 12.1, 11.6, and 11.6 kg/d, respectively. BW (718 kg) and BCS (3.38) of the dams neither differed by treatment nor was there a treatment by week interaction. Yield (6.1 kg) and Brix reading (26.1) of the colostrum was similar among treatments. Calf birth weight (42.3 kg) also was similar among treatments. Immune function measures will include concentrations of IgG in both the dam and calf and lymphocyte blastogenesis in the calf. Other than for DMI on dams, physical measures were similar amond treatments, but further investigation on immune function will provide addition insight to whether ω-6:ω-3 can impact fetal and neonate development.

Key Words: immunity, fatty acids, prepartum

M15 Empirical modelling of vitamin B_{12} duodenal flow in lactating dairy cows. V. Brisson*[1], C. L. Girard[2], J. A. Metcalf[3], D. S. Castagnino[3], J. Dijkstra[4], and J. L. Ellis[1], [1]*University of Guelph*, *Guelph, ON, Canada*, [2]*Agriculture and Agri-Food Canada*, *Sherbrooke, QC, Canada*, [3]*Trouw Nutrition Canada*, *Guelph, ON, Canada*, [4]*Wageningen University and Research*, *Wageningen, the Netherlands*.

Unlike other B vitamins, vitamin B_{12} is not found in plants and is produced only by bacteria. Therefore, supply to the dairy cow, unless provided via supplementation, will mainly be the result of B_{12} manufactured by ruminal microbes. The duodenal flow of B vitamins therefore represents the amount of vitamin available for absorption by the ruminant, which can be used for essential metabolic functions and milk production. However, diet composition may affect ruminal synthesis and the resulting duodenal flow (DF) of vitamin B_{12}, due to alterations to fermentation and ruminal conditions. Therefore, the objective of this study was to conduct a meta-analysis describing how diet composition affects DF of vitamin B_{12}. Data were collected from 340 individual lactating cows involved in 16 published studies. Saved diet and duodenal samples from these studies were subsequently reanalyzed for B vitamin content to create the database used in the present study. Potential driving variables considered included (DM basis) dietary organic matter (%), NDF (%), starch (%), crude protein (%) and DMI (kg/d). The meta-analysis was conducted in 3 steps, followed by statistical evaluation of the resulting empirical models. A Spearman correlation matrix was constructed between all potential driving variables to assess for collinearity between X variables, and guide model creation. Then, using Cook's distance statistic (Proc MIXED), outliers were determined and removed. Finally, a suite of potential models (with study treated as a random effect) were developed in GLIMMIX. Where models were statistically significant, evaluation was completed using root mean square prediction error (RMSPE) and concordance correlation coefficient (CCC) to determine the sources of error. The best performing model was: B12DF (mg/d) = −7.87 (±2.46) + 0.29 (±0.056) × DietNDF(%) + 0.44 (±0.042) × DMI (kg/d); RMSPE: 41.1%, CCC: 0.268. In conclusion, DF of B_{12} was positively impacted by both the overall DMI and the dietary NDF content of the diet. This information may be used to better understand supply of vitamin B_{12} to the modern dairy cow, in relation to requirements, to improve milk production efficiency.

Key Words: vitamin B_{12}, meta-analysis, duodenal flow

M16 Genome-wide association study and functional analyses of clinical and subclinical ketosis in Holstein cattle. R. A. N. Soares*, G. Vargas, F. S. Schenkel, and E. J. Squires, *University of Guelph*, *Guelph, ON, Canada*.

Ketosis affects high yielding cows and it is one of the most frequent metabolic diseases in dairy cows causing high economic losses. Therefore, finding genetic markers for gene variants associated with resistance to ketosis is of interest to genetically select for less susceptible cows. The aim of this study was to identify and investigate genomic regions associated with clinical and subclinical ketosis in Holstein cattle. To achieve this, weighted single step genome-wide association study (wssGWAS) was performed considering 4 traits: clinical ketosis in first (CK1) and later lactations (from 2 to 5; CK2), and subclinical ketosis in first (SCK1) and later lactations (from 2 to 5; SCK2). The estimated breeding values (EBV) from 77,277 cows and 7,704 bulls were de-regressed and used as pseudo-phenotypes in the GWAS. The wssGWAS model was: $y^* = \mu + Z_a\alpha + e$, where y^* is the vector of pseudo-phenotypes; μ is the overall mean; Z_a is a matrix that relates animals to pseudo-phenotypes; α is the vector of additive genetic effects and e is the vector of random residuals. The top-20 genomic regions explaining the largest proportion of the genetic variance were investigated for putative genes associated with the traits through functional analyses. Regions of interest were identified in chromosomes 2, 5 and 6 for CK1, 3, 6 and 7 for CK2, 1, 2 and 12 for SCK1 and 20, 11 and 25 for SCK2. The highest proportion of genetic variance explained by a region was located on BTA2 for SCK1. The highlighted genes potentially related to clinical and subclinical ketosis included *ACAT2* and *IFG1.* Enrichment analyses of the candidate genes for the traits showed molecular functions and biological processes that are associated with fatty acid metabolism, synthesis and degradation of ketone bodies and inflammatory response. Several genomic regions and SNPs related to susceptibility to ketosis in dairy cattle, which were previously described in other studies were confirmed here. In addition, some novel potential regions were found that would warrant further investigation on their potential association with clinical and subclinical ketosis.

Key Words: association study, ketones, negative energy balance

M17 Mammary blood vessel development in response to estradiol administration in heifer calves. N. R. Hardy*[1], K. M. Enger[1], M. L. Eastridge[2], L. E. Moraes[2], and B. D. Enger[1], [1]*The Ohio State University, Department of Animal Sciences, OARDC*, *Wooster, OH*, [2]*The Ohio State University, Department of Animal Sciences*, *Columbus, OH*.

Mammary blood flow is central to mammary growth, development, and productivity, but development of the vasculature network is poorly understood. The objective of this study was to determine how the vascular system adapts to mammary growth by inducing different levels of mammary growth and examining 2 regions of the mammary tissue. Holstein heifer calves received 12 daily injections on the days immediately preceding euthanasia at 82 d of age. Treatments were control (n = 4, CON), short-term estradiol (n = 4, SHORT), and long-term estradiol (n = 4, LONG). CON calves received corn oil injections while SHORT calves received 9 injections of corn oil followed by 3 injections of estradiol; LONG calves received 12 estradiol injections. Mammary tissues were collected from the center and edge parenchymal regions of all right rear mammary glands to quantify the tissue area of various tissue structures, the percentage of proliferating epithelial cells, and the number and form of blood vessels. Data were analyzed using PROC MIXED with the fixed effects of calf treatment and parenchymal region, and calf nested within treatment as a random effect. Results showed LONG calves had a greater tissue area occupied by epithelium (34.0% ± 1.5, $P < 0.05$) than CON and SHORT calves (21.4% and 23.0% ± 1.5, respectively), while CON and SHORT calves were similar. Edge parenchyma had a greater percentage of proliferating epithelial cells than center parenchyma across all treatment groups. Within the edge region, LONG calves had the greatest percent of proliferating epithelial cells ($P < 0.05$). Blood vessel number per unit of tissue area was greater in center than edge parenchyma (395 vs 295 ± 19 vessels/mm^2, $P < 0.001$, respectively); the corresponding vessel surface area/unit of tissue area followed this same pattern (23,140 vs 18,166 ± 1088 μm/mm^2, $P < 0.001$). These vessel measures were not affected by estradiol treatment ($P > 0.1$). These results show there is a large difference in blood vessel number in the center versus the edge parenchyma and estradiol treatment elicits mammary growth but not necessarily increases in blood vessel formation.

Key Words: angiogenesis, growth, endothelium

M18 Effects of physically effective undigested neutral detergent fiber and rumen fermentable starch on lactation performance and total tract digestibility of lactating cows. K. M. Smith*[1], A. Obata[2], K. Hirano[2], H. Uchihori[2], S. Y. Morrison[1], J. W. Darrah[1], H. M. Dann[1], C. S. Ballard[1], M. D. Miller[1], and R. J. Grant[1], [1]*Miner Institute*, *Chazy, NY*, [2]*ZEN-NOH*, *Tokyo, Japan*.

Multiparous cows (n = 16) were used in a 4 × 4 replicated Latin square design to evaluate the effect of feeding different dietary concentrations of 240-h physically effective undigested neutral detergent fiber (peuNDF240) and rumen fermentable starch (RFS) on intake, milk yield and composition, and total-tract digestibility (TTD). Diets differed in peuNDF240 and RFS by inclusion of different corn silage hybrids and cornmeal amount. Treatments were 1) low peuNDF240 (6.4%DM), low RFS (16.7%DM; LULR) 2) low peuNDF240 (6.1%DM), high RFS (19.2%DM; LUHR) 3) high peuNDF240 (8.6%DM), low RFS (16.9%DM; HULR) 4) high peuNDF240 (8.0%DM), high RFS (19.0%DM; HUHR). On d 19–28 of each 28-d period, samples for dry matter intake (DMI), milk yield, milk composition, behavior, rumen pH, rumen fluid, and TTD were taken. Data were summarized by period and analyzed with model effects of diet, period, and replicate using MIXED procedure of SAS (v.9.4). Cow within replicate was a random effect. No significant treatment differences ($P > 0.05$) were observed for (mean ± SEM) DMI, total chewing time (809 ± 14 min/d), daily mean rumen pH (6.1 ± 0.1), total ammonia (7 ± 10 mg/dL), and total VFA (125 ± 3 mM). Cows fed LULR had higher 3.5% fat-corrected milk (FCM) compared with cows fed LUHR with HU diets being intermediate (Table 1). The LULR diet had greater TTD of neutral detergent fiber (NDF) compared with HU diets, and LU diets had lower TTD of starch compared with HU diets. Overall, this data shows that there is a balance when adding fermentable starch to diets with highly digestible fiber in the physically effective fraction, and lesser effect in diets with less digestible fiber in the physically effective fraction.

Table 1 (Abstr. M18).

Item	LULR	LUHR	HULR	HUHR	SEM	*P*-value
DMI, kg/d	29.7	29.4	29.4	29.2	0.7	0.56
Milk, kg/d	53.1^{a}	52.0^{ab}	51.2^{b}	51.5^{ab}	1.3	0.04
Fat, %	3.59	3.48	3.74	3.60	0.08	0.06
FCM, kg/d	53.8^{a}	51.5^{b}	52.9^{ab}	52.2^{ab}	1.3	0.05
TTD NDF, % DM	62.0^{a}	60.1^{ab}	58.6^{b}	57.6^{b}	0.59	0.006
TTD starch, %DM	98.2^{b}	98.5^{b}	98.9^{a}	98.7^{a}	0.14	0.002

^{ab}Least squares means within row without common superscript differ ($P \leq 0.05$).

Key Words: undigested fiber, rumen fermentable starch, physically effective fiber

M19 Effects of probiotics, enzymes, and yeast combinations on ruminal fermentation in a dual-flow continuous culture system. S. Bennett*[1], J. A. Arce-Cordero[1], V. L. N. Brandao[1], J. R. Vinyard[1], B. Agustinho[2], H. F. Monteiro[1], L. Tomaz[3], R. Lobo[1], and A. P. Faciola[1], [1]*University of Florida*, *Gainesville, FL*, [2]*State University of Maringá*, *Maringá, Paraná, Brazil*, [3]*Sao Paulo State University*, *Sao Paulo, Sao Paulo, Brazil*.

The objective of this study was to evaluate the changes in ruminal fermentation when the diet is supplemented with different combinations of probiotics, enzymes, and live yeast. Our hypotheses were (1) inclusion of additives would increase nutrient digestibility and volatile fatty acid concentration, and (2) increasing additive doses would lead to further im-

provements in digestibility. Diets were randomly assigned to 8 fermentors in a replicated 4 × 4 Latin square with four 10-d experimental periods, consisting of 7 d for diet adaptation and 3 d for sample collection. Diets contained 50:50 forage:concentrate and fermentors were fed 106 g of dry matter per day divided equally between 2 feeding times. Treatments were control; bacterial culture/enzyme blend (1.7 mg per day); bacterial culture/enzyme blend with live yeast (49.76 mg per day); and double dose of the bacterial culture/enzyme blend with live yeast treatment (99.53 mg per day). The bacterial culture/enzyme blend contained 5 strains of live bacteria with a concentration of 10^{10} cfu (*Lactobacillus animalis, Propionibacterium freudenreichii, Bacillus lichenformis, B. subtillis*, and *Enterococcus faecium*) and 3 enzymes (amylase, hemicellulase, and xylanase). The yeast component was *Saccharomyces cerevisiae.* On d 8 and 9, samples were collected for pH, redox, volatile fatty acid (VFA), lactate, NH_3-N and digestibility measurements. Statistical analysis was performed using the GLIMMIX procedure of SAS with fermentor, square and period as random effects. Significance was declared at $P \leq 0.05$. No effects were observed for pH, redox, NH_3-N, acetate, isobutyrate, valerate, total VFA, acetate:propionate, nutrient digestibility or N utilization. Furthermore, no effects were observed for the hourly propionate molar proportion. Within the pooled effluent samples, butyrate increased with the inclusion of additives when compared with the control while propionate had a tendency to decrease. In conclusion, the addition of probiotics, enzymes, and yeast to the diet increased butyrate concentration.

Key Words: butyrate, *Lactobacillus*, *Saccharomyces cerevisiae*

M20 Effects of precision feeding Holstein and Jersey heifers an increasing poultry fat inclusion on rumen fermentation parameters. S. M. Hussein*[1], S. Twyman[1], M. Toledo[1], O. Thomas[1], J. Echesabal[1], R. M. Stockler[2], M. J. Aguerre[1], and G. J. Lascano[1], [1]*Clemson University*, *Clemson, SC*, [2]*Auburn University*, *Auburn, AL*.

Fat inclusion can increase energy density of diets fed to ruminants, therefore reducing dry matter intake (DMI) required to meet caloric demands, however detrimental effects to rumen fermentation have been reported. There is evidence that not all fat sources have this negative response. Diets used for precision-feeding are more nutrient dense, allowing an increase in energy and nutrient utilization efficiency while decreasing nutrient loss. We hypothesized that including poultry fat (PF) would reduce intake without compromising rumen fermentation in dairy heifers. Four Holstein and 4 Jersey cannulated heifers were randomly assigned to 4 treatments, included 55% forage diet with 4 increasing PF inclusion starting with a basal concentration of fat in the diet [3% fat (0% PF); 5% fat (2% PF); 7% fat (4% PF); and 9% fat (6% PF)]. Treatments administered according to a split-plot, 4 × 4 Latin square design for 4 periods of 21 d [(16 d adaptation, (5 d sampling; rumen sampling were collected on the last day of each period)]. Data were analyzed using the MIXED procedure of SAS with fixed effects of period, breed, PF inclusion, breed × PF interaction, and a random effect of heifer (breed) and repeated measures when needed. There were no differences on rumen VFA between the Holstein and Jersey-group. Total VFA concentration and acetate molar proportion decreased linearly with the increased level of PF inclusion. Concurrently, there was a linear increase in propionate molar proportion resulting in a linear reduction on the A:P ratio with PF incremental inclusion. Iso-butyrate molar proportion increased linearly as PF increased. Mean rumen pH was not affected by breed, but the PF inclusion showed a linear increase on rumen pH ($P < 0.03$). Rumen NH_3-N concentration was not affected by breed, but PF inclusion resulted in an increasing linear trend on rumen NH_3-N ($P = 0.10$). These results suggest that Holstein and Jersey heifers had a similar pattern and rate of rumen fermentation and that increasing PF inclusion in precision feeding program is affecting rumen fermentation pattern mainly by reducing the A:P.

Key Words: precision feeding, poultry fat, heifer

M21 Effects of feeding rumen-protected lysine during the transition period on postpartum immunometabolic gene expression and morphology of uterine samples of Holstein cows. A. Guadagnin*[1], L. Fehlberg[1], B. Thomas[1], Y. Suginomoto[2], I. Shinzato[2], and F. Cardoso[1], [1]*University of Illinois, Department of Animal Sciences*, *Urbana, IL*, [2]*Ajinomoto Co. Inc*, *Tokyo, Japan*.

Methionine has an impact on the reproductive immune function and metabolism of dairy cows. However, whether lysine plays a similar role is unknown. We aimed to determine the effects of feeding rumen-protected Lys (RPL, AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) pre- (PRE, 0.54%DM of TMR) and/or postpartum (POS, 0.395%DM of TMR) on mRNA gene expression profiles of endometrial samples of Holstein cows. Seventy-three (n = 73) multiparous Holstein cows were randomly assigned to 1 of 4 dietary treatments in a randomized, complete block design with a crossover of a diet with RPL (L) or without (C). Treatments consisted of TMR with RPL pre- and postpartum (LL), with RPL pre- and without RPL postpartum (LC), without RPL pre- and with RPL postpartum (CL), and without RPL pre- and postpartum (CC). Endometrial tissue samples were collected through uterine biopsy at 28 d relative to calving and analyzed for expression of genes related to inflammation and protein metabolism, and stained with hematoxylin and eosin for morphology evaluation. Data collected were analyzed using the MIXED procedure in SAS. There was a tendency for an effect of POS ($P = 0.06$) on the gene expression of mucin 1 mRNA (MUC1) and mucin 4 mRNA (MUC4), in which cows in L postpartum had greater expression of MUC1 (1.15 ± 0.48) and MUC4 (1.05 ± 0.43) than cows in C (0.83 ± 0.48 and 0.22 ± 0.43, respectively). A PRE x POS interaction was observed for the expression of interleukin-10 mRNA (IL10), where cows in LL (11.31 ± 1.40) had greater ($P < 0.01$) expression of IL10 than cows in CC (4.57 ± 1.40), CL (4.00 ± 1.40), or LC (6.06 ± 1.40). There was no difference in the number of endometrial glands ($P = 0.20$) nor on the number of cells per gland ($P = 0.14$). In conclusion, feeding RPL postpartum impacts uterine immune response, through increasing the expression of mRNAs that encode glycoproteins involved in the protection and renewal of epithelial cells, and also increasing anti-inflammatory cytokine IL10 mRNA expression.

Key Words: gene expression, lysine, mucin

M22 Effects of ruminally degraded protein and starch on gluconeogenic precursors in lactating cows. A. Hruby*, L. Campos, M. Li, J. Prestegaard, and M. Hanigan, *Virginia Tech*, *Blacksburg, VA*.

In lactating dairy cows, gluconeogenesis is vital to maintain glucose (Glu) levels for milk production. Propionate (Pr) produced by ruminal fermentation is the primary precursor for Glu synthesis, however, lactate and amino acids (AA) also contribute. The objectives of this study were to

Table 1 (Abstr. M22). Least squares means of metabolite and AA concentrations in pooled blood plasma samples[1]

	Low RDP		High RDP			P-value[2]		
Item	LS	HS	LS	HS	SE	RDP	S	RDP × S
Propionate entry rate (g/h)	51.4	24.2	25.1	52.8	6.80	0.87	0.97	<0.01
Propionate (μ*M*)	32.0	32.2	28.0	34.0	3.10	0.58	0.12	0.16
Lactate (m*M*)	0.619	0.660	0.650	0.500	0.050	0.12	0.18	0.03
Ala (μ*M*)	327	343	328	314	19.1	0.55	0.97	0.55
Ile (μ*M*)	203	160	145	141	12.3	<0.01	0.03	0.07
Met (μ*M*)	22.9	27.1	22.8	28.4	1.19	0.56	<0.01	0.46
Pro (μ*M*)	104	113	89.1	102	6.30	<0.01	<0.01	0.56
Ser (μ*M*)	93.7	108	91.8	103	6.90	0.59	0.07	0.81
Val (μ*M*)	353	301	302	287	24.5	0.04	0.05	0.22

[1] S = starch; LS = low starch; HS = high starch.

[2] Significance declared at $P<0.05$.

assess the relative contributions of these 3 substrates to gluconeogenesis when Pr and AA supplies are manipulated and to assess the validity of determining Pr production via isotope dilution in the blood pool. Two multiparous and 4 primiparous Holstein cows (247 ± 26 and 166 ± 61 DIM, respectively) were randomly assigned to 1 of 4 treatments. Treatments were arranged in a 2 × 2 factorial design consisting of high and low dietary concentrations of ruminally degraded protein (RDP), and high (HS) and low (HS) dietary concentrations of starch. Treatments were applied in a 4 × 4 Latin Square with 4, 14-d periods. [2–13C]-Pr was ruminally infused on d12. Jugular infusions of [1,2–13C]-Glu, [1–13C]-lactate, and [2–13C]-Pr were administered on d13 and [U-13C]-AA on d14. Jugular vein samples were taken during and following each infusion and assessed for substrate concentrations and isotopic enrichment. Blood samples were pooled and assessed for lactate, Pr, Glu, and AA concentrations. Met and Pro concentrations were greater for diets containing high starch; further, the latter was lower with high RDP (Table 1). Lactate concentrations were greater for the high RDP, HS treatment, and Pr concentrations tended to be greater with HS. Further, Pr appearance in blood through direct jugular infusions was assessed by isotope dilution in response to varying RDP and starch. Data from this experiment will allow preparation of an improved model for gluconeogenesis in lactating cows.

Key Words: stable-isotope, VFA, propionate

M23 Stimulation of microbial protein synthesis by branched-chain volatile fatty acids in dual-flow cultures varying in forage and PUFA concentrations. K. E. Mitchell*[1], B. A. Wenner[1], C. Lee[1], M. T. Socha[2], and J. L. Firkins[1], [1]*The Ohio State University*, *Columbus, OH*, [2]*Zinpro Corporation*, *Eden Prairie, MN.*

Branched-chain amino acids (BCAA) are deaminated by amylolytics to branched-chain VFA (BCVFA), which are growth factors for cellulolytic bacteria that cannot degrade or synthesize their own BCAA. Our objective was to determine the dietary conditions that would increase the uptake of BCVFA. Increasing forage would increase cellulolytic bacterial abundance and their stimulation by BCVFA as growth factors. However, in a low forage diet, amylolytic bacteria should outcompete for BCVFA. Supplemental PUFA should inhibit cellulolytic bacteria growth, but additional BCVFA should minimize that inhibition. Supplementation of BCVFA could therefore increase efficiency of microbial protein synthesis in both low forage (LF), high forage (HF), and high PUFA diets. The study was an incomplete block design with 8 dual-flow continuous cultures used in 4 periods with treatments (n = 4) arranged as a 2 × 2 × 2 factorial. The factors were: HF or LF (67 or 33%), without or with supplemental corn oil (CO, 3% DM), and without or with 2.15 mmol/d each of isovalerate, isobutyrate, and 2-methylbutyrate (MB). The isonitrogenous diets consisted of 33:67 alfalfa: orchard grass pellet, and was replaced with ground corn and soybean meal in the LF diet. Data were analyzed with PROC MIXED in SAS (v. 9.4, SAS Institute 2015) with random effects of period and fermenter and fixed effects of diet, CO, BCVFA, and their interactions. The main effect of supplementing BCVFA increased (P = 0.10) NDF digestibility (NDFd) by 2.97% units, and CO increased (P = 0.07, for interaction) NDFd by 6.46% units in LF diets. BCVFA and HF increased ($P < 0.03$) bacterial N by 1.54 and 1.84 g/kg OM truly degraded, respectively. Total BCVFA net production was lower ($P < 0.01$) with HF compared with LF. When CO and BCVFA were supplemented with LF, total BCVFA net production decreased (P = 0.10, for interaction). When BCVFA was supplemented with CO, MB net production decreased (P = 0.08, for interaction) with HF, but even more with LF. Providing supplemental BCVFA improved efficiency of cellular growth of cellulolytics and therefore NDFd, which should improve feed efficiency in dairy cows.

Key Words: branched-chain VFA, cellulolytic, PUFA

M24 Effects of Enogen Feed corn silage and corn grain on nutrient digestibility, production, and enteric methane emission in lactating cows. L. Rebelo*[1], C. Lee[1], W. Weiss[1], and M. Eastridge[2], [1]*The Ohio State University*, *Wooster, OH*, [2]*The Ohio State University*, *Columbus, OH.*

Increasing rumen starch digestibility can improve production and feed efficiency. Corn that was developed to contain high α-amylase expression (Enogen Feed, Syngenta Seeds, LLC, Downers Grove, IL) was evaluated on nutrient digestibility, production, and CH_4 emission in lactating cows. Enogen and isoline corn were grown and harvested for corn silage (CS) and corn grain (CG). Fifteen cows (6 ruminally cannulated; 9 non-cannulated) were used in a replicated 3 × 3 Latin square design (5 orthogonal squares; 14-d adaptation and 14-d sampling in each period). Dietary treatments were: isoline CS and isoline CG (CON); Enogen CS and isoline CG (ECS); Enogen CS and Enogen CG (ECSCG). The diets included 48% CS and 19% CG (DM basis) and contained 15.5% CP, 31% NDF, and 29% starch (DM basis). A 4-d total collection of feces was conducted for nutrient digestibility with cannulated cows, and CH_4 was measured in 8 equally spaced times throughout 3 d using Greenfeed (C-Lock Inc.) from non-cannulated cows. Production was obtained from all cows. Data were analyzed using the MIXED procedure of SAS with treatment and period as fixed and cow and cow within square as random effects. Total-tract digestibility of DM, OM, CP, and NDF were not different among treatments. Compared with CON, ECS and ECSCG increased DMI (26.7 and 26.6 vs. 25.1, respectively; $P < 0.01$). Milk yield increased only for ECS compared with CON (36.5 vs. 33.1 kg/d; $P = 0.03$). Energy-corrected milk was not affected (34.5, 36.4, and 35.6 kg/d for CON, ECS, and ECSCG, respectively). Milk protein yield was greater ($P = 0.02$) for ECS vs. CON and ECSCG (no difference between CON and ECSCG). Milk fat content tended to be lower ($P = 0.06$) for ECS vs. CON and ECSCG (no difference between CON and ECSCG). Methane yield decreased (11.5 vs. 13.5 g/kg DMI; $P = 0.04$) only for ECS vs. CON. In conclusion, ECS increased milk and protein yields and lowered CH_4 yield compared with CON, possibly because of greater starch availability in the rumen. However, ECSCG had only numerical effects on milk and ECM yields compared with CON.

Key Words: Enogen corn grain, Enogen corn silage, production

M25 Cooling dairy calves pre- and postnatally increases circulating serotonin and modulates immune function. M. G. Marrero*, B. Dado-Senn, S. L. Field, and J. Laporta, *University of Florida*, *Gainesville, FL.*

Heat stress suppresses dairy calves' ability to develop optimum immune responses predisposing them to increased morbidity and mortality. We investigated the effects of providing heat stress abatement across pre- and postnatal periods on dairy calf immune system development. We hypothesized that pre-weaned calves receiving heat stress abatement will have increased humoral immunity and circulating serotonin (5-HT, an immunomodulator). To test this, calves born to heat stressed (HS) or cooled (CL) dams during late gestation (~46 d) were then exposed to postnatal HS or CL environments (56 d) resulting in 2 treatments: HSHS and CLCL; n = 6/group. Postnatal CL was achieved using fans providing wind speed ≥2 m/s. All calves were fed 4 L pooled colostrum at birth. Blood samples were collected weekly (d0 to 56) to measure leukocyte populations, 5-HT and IgG concentrations. On d47 a 24h in vitro LPS-challenge (0, 0.5, 1.0, 5.0 µg/mL dose) was performed. On d0, 21 and 42 blood was collected using Tempus RNA tubes for gene expression analysis of heat shock proteins (*HSP70* and *HSP72*), transforming growth factor β (*TGFB*), toll-like receptors 2 and 4 (*TLR2* and *TLR4*), interferon gamma (*IFNG*), T-cell transcription factors (*GATA3*, *FoxP3*, *TBX21* and *RORcy*), 5-HT receptors 2A and 2B, 5-HT transporter (*SERT*) and tryptophan hydroxylase (*TPH1*). Data were analyzed using mix-model ANOVAs with treatment, day and interaction as fixed effects and ID as random. CLCL calves had greater *TPH1* expression at birth ($P = 0.005$) and greater circulating 5-HT ($P = 0.04$), however, both groups had greater 5-HT concentrations after LPS-challenge in vitro ($P = 0.01$). Although there were no differences in neutrophil, monocyte and lymphocyte counts ($P > 0.11$), CLCL calves had greater gene expression of *HSP70*, *FoxP3*, *TBX21*, *TGFB*, *TLR4* and *5-HT2A* ($P < 0.04$). Notably, there was a treatment by day interaction whereby IgG concentrations were higher in the CLCL calves from d35 and thereafter ($P = 0.01$). Heat stress abatement increases circulating serotonin and has the potential to improve adaptive and humoral immune development in pre-weaned dairy calves.

Key Words: serotonin, immunology, heat stress

M26 Assessment of the colon transcriptome changes from pre- to post-weaning in calves fed elevated levels of milk. J. K. van Niekerk*[1], M. Middeldorp[2], L. L. Guan[1], and M. A. Steele[1,3], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta*, *Edmonton, AB, Canada*, [2]*Animal Nutrition Group, Wageningen University & Research*, *Wageningen, the Netherlands*, [3]*Department of Animal Biosciences, Animal Science and Nutrition, University of Guelph*, *Guelph, ON, Canada*.

In addition to rumen fermentation, colon fermentation plays an important role in providing energy to ruminants as well. The aim of this study was to assess colon epithelial gene expression changes in calves from pre- to post-weaning and to determine if these changes are associated with calf starter (CS) intake, colon mucosa thickness, fecal starch and serum inflammatory marker concentrations in calves fed at a high milk replacer (MR) feeding rate. Six Holstein calves were fed MR (150 g/L) at 15% of BW and volumes were adjusted weekly according to BW. Calves were weaned by reducing milk allocation by 50% during wk 6. Serum was collected for acute phase protein analysis and fecal matter for starch analysis weekly from wk 4 to 12. Endoscopic biopsies of the colon were performed for histological measurements (wk 5, 6, 7, 8 and 12) and transcriptomic analysis (wk 5, 7 and 12). Phenotypic trait data were analyzed using GLIMMIX procedure of SAS with wk as a fixed effect and calf as a random effect. Fecal starch was higher ($P = 0.0002$) at wk 9 compared with wk 4, 5 and 6 and colon mucosa thickness tended to be decreased ($P = 0.08$) at wk 8 compared with wk 5. The transcriptome analyses uncovered 10 genes (3 up- and 7 downregulated) differentially expressed (DE) during short-term adaptation (wk 5 to 7) while 65 genes (38 up- and 37 downregulated) were DE during long-term adaptation (wk 5 to 12). Weighted gene co-expression network analysis identified a gene module (517 genes) that had a negative correlation (Pearson correlation) with fecal starch ($P = 0.01$) and CS intake ($P < 0.001$) and a positive correlation with colon mucosa thickness ($P = 0.02$). There were 13 DE genes that overlapped with the significant co-expressed gene module and most of these genes (*AQP8, SLC7A8, SLC13A2, SLC9A3, SLC6A14, SLC40A1* and *S100A12*) were involved in nutrient transport and inflammatory response. This is the first study to provide evidence of these genes being expressed in the colon of young ruminants and their change in expression suggests that they may be used as future gene markers to evaluate nutrient absorption and gut and immune function.

Key Words: colon, transcriptome, mucosa thickness

M27 Association between haptoglobin concentrations and disease incidence, milk production, and reproductive performance at the cow and herd level. A. L. Kerwin*[1], D. V. Nydam[2], W. S. Burhans[3], S. K. Wall[4], K. M. Schoenberg[4], K. L. Perfield[4], and T. R. Overton[1], [1]*Department of Animal Science, Cornell University*, *Ithaca, NY*, [2]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University*, *Ithaca, NY*, [3]*Dairy-Tech Group*, *South Albany, VT*, [4]*Elanco US Inc*, *Greenfield, IN*.

The objectives of this prospective observational study were to: 1) evaluate the association between elevated haptoglobin (HP) on 305 d mature equivalent milk at 120 DIM (ME305), conception within 150 DIM and conception at first service and 2) identify the HP herd-alarm level that is associated with herd-level changes in disease incidence (DI; displaced abomasum or ketosis), ME305, 21-d pregnancy rate (PR) and conception rate (CR) at first service. Plasma samples were collected from 868 cows, 0 to 12 DIM, across 72 herds, and were analyzed for HP (University of Guelph Animal Health Laboratory). Haptoglobin was dichotomized based on thresholds associated with increased DI (range: 0.55 to 1.52 g/L). Mixed effects models were used for the ME305 and herd-alarm level outcomes (PROC MIXED, SAS 9.4). Conception within 150 DIM was analyzed by a Cox proportional hazards model (PROC PHREG). Conception at first service was analyzed with a mixed effects Poisson regression (PROC GENMOD). The herd-alarm level with the smallest proportion of cows with elevated HP and the lowest HP threshold that resulted in the lowest P-value and largest change in the outcome of interest was kept in the final model. The initial proportion evaluated was 30% and it was lowered by 5% increments. Cows with HP ≥ 0.55 g/L produced 898 kg less ME305 ($P < 0.01$) and had a 25% (hazard ratio = 0.75; $P < 0.01$) decreased risk of conception by 150 DIM. Cows with HP ≥ 1.52 g/L were 0.60 times as likely to conceive at first service (risk ratio = 0.60; $P = 0.01$). The herd-alarm level associated with DI was defined as ≥ 20% of cows having HP ≥ 0.55 g/L, resulting in a 5.8% increase in DI ($P = 0.01$). The herd-alarm level associated with PR was defined as ≥ 10% of cows having HP ≥ 1.52 g/L, resulting in a 2.5% decrease in 21-d PR ($P = 0.09$). The herd-alarm level associated with CR at first service was defined as ≥ 20% of cows having HP ≥ 1.52 g/L, resulting in a 6.4% decrease in CR at first service ($P = 0.09$). There was not a herd-alarm level associated with ME305. These results indicate that HP can be used as a herd health-monitoring tool at the cow and herd-level.

Key Words: inflammation, haptoglobin, transition cow

M28 Bacteria count differences in used bedding and bulk tank milk based on bedding type in five New York dairy farms. V. Alanis*[1], M. Zurakowski[2], D. Pawloski[2], D. Nydam[1], and P. Ospina[1], [1]*Cornell University*, *Ithaca, NY*, [2]*Cornell University*, *Cobleskill, NY*.

The objectives were to evaluate the differences in bacteria counts between bulk tank milk and 5 different used bedding types (manure solids, paper fiber, straw, recycled sand, and sand) from lactating pens from 5 New York Dairies. Sampling occurred monthly over one year (July 2018–2019). Bedding was collected making sure to avoid manure, from 3 to 5 representative stalls following a strict standard operating procedure to minimize sampling variability, and a bulk tank milk sample was collected on the same day. Bedding (cfu/g) and bulk tank milk (cfu/mL) were cultured for coliform, non-coliforms, and *Streptococcus* spp. counts. In milk, *Staphylococcus* spp. counts were also reported. The Kruskal-Wallis test was used to evaluate bacterial count differences in individual bedding and milk samples. The only bacteria count difference in milk was *Staphylococcus* spp. where farm bedding with paper fiber had the highest counts and sand had the lowest ($P = 0.01$). There were no differences in other bacteria counts in milk ($P > 0.1$). Although there were significant statistical differences between bedding type with organic bedding having slightly higher bacteria counts, overall, organic bedding showed similar bacterial counts than inorganic bedding (Table 1). However, it is important to note that farms using organic bedding were re-bedding more frequently than those using sand.

Key Words: bedding, bacterial counts, milk quality

M29 Preference, feeding behavior, and in vitro fermentation characteristics of pelleted feeds containing different binders in growing primiparous Holstein cows. H. T. Hong*[1], H. C. Hung[1], K. R. Kerner[1], A. L. Peterson[1], S. Burkhardt[2], A. H. Laarman[1], and P. Rezamand[1], [1]*University of Idaho*, *Moscow, ID*, [2]*Sustainable Fiber Technologies*, *Renton, WA*.

Objectives of this study were to test preference, feeding behavior, and rumen fermentation characteristics of a new feed binder, a thermo-chemically treated lignin product. Five pelleted feeds were prepared including a negative control containing neither molasses nor the new product (NCTL), a positive control containing Ameri-Bond 2X at 2.1% dry matter

Table 1 (Abstr. M28).

Bacteria group count	Used bedding type				
	Manure solids n=44	Paper fiber n=70	Straw n=18	Recycled sand n=60	Sand n=45
Bedding samples^{1}					
Coliforms	6.7±0.9 (6.4–6.9)^{a}	5.9±0.7 (5.8–6.1)^{b}	6.7±0.6 (6.4–6.9)^{a}	4.9±0.7 (4.8–5.1)^{c}	4.9±0.7 (4.7–5.2)^{c}
Non-coliforms$^{(n)2}$	8.1±0.4 (7.8–8.2)^{a}	6.5±1.0 (6.2–6.5)$^{b(7)}$	7.1±0.6 (6.8–7.3)^{b}	5.8±0.7 (5.6–5.8)$^{c(6)}$	5.9±0.6 (5.7–6.1)$^{c(10)}$
Streptococcus spp.	9.1±0.3 (8.9–9.2)^{a}	9.2±0.5 (9.0–9.3)^{a}	9.5±0.5 (9.2–9.7)^{b}	8.3±0.6 (8.1–8.4)^{c}	8.5±0.9 (8.2–8.8)^{d}
Bedding frequency	Every day	Twice weekly	Every day	Weekly	Weekly
Bulk tank samples^{3}					
Staphylococcus spp.	2.3±0.6 (1.8–2.8)^{AB}	3.3±0.3 (2.9–3.6)^{A}	2.7±0.6 (2.0–3.4)^{AB}	2.6±0.4 (2.2–2.9)^{AB}	2.2±0.4 (1.9–2.5)^{B}

$^{a–d}$Means with different lowercase superscripts within a row differ ($P < 0.0001$).

^{A,B}Means with different uppercase superscripts within a row differ ($P < 0.01$).

[1] Mean ± SD, (95% CI) bacteria counts ($\log_{10}$ cfu/g) in bedding over a year.

[2] Where (n) indicates number of samples removed due to no growth culture result.

[3] Mean ± SD, (95% CI) bacteria counts ($\log_{10}$ cfu/mL) in bulk tank over a year.

basis (DM, PCTL). Three pelleted feeds that contained the new binder were low (1.6% DM, LOW), medium (3.2% DM, MED), high (4.8% DM, HIGH). Ten Holstein heifers were placed in individual stalls and given one feed, for one hour, to determine the acceptance, intake, and feeding behavior regarding each feed. To determine the preference, cows were given 2 feeds (a reference diet, NCTL; and another feed) in 2 separate buckets with rotations for one hour. Intake was measured and preference was calculated using the following: Preference % = (Test diet) / (Test diet + Reference diet) × 100%. Pelleted feeds were placed in the rumen fluid in vitro for 0, 4, 8, 12, 18, 24, 48 and 72 h. Degradation of dry matter (DM), organic matter (OM), NDF, and ADF were analyzed. Data were analyzed using the PROC GLIMMIX (SAS v. 9.4) with significance declared at $P \leq 0.05$. Acceptance of LOW was the highest ($P = 0.001$). Cows spent more time eating on LOW than other feeds ($P = 0.001$) and less time wandering on NCTL and LOW ($P = 0.045$), but no difference observed in rumination and drinking time. Intake at each approach was greater for MED which did not differ from LOW or HIGH ($P = 0.03$). The number of times that cows approached and ate feed for LOW was the highest but it did not differ from NCTL or HIGH ($P = 0.025$). Cows preferred LOW over other feeds ($P = 0.001$). The degree of DM degradation for PCTL, LOW, and MED were greater than that of NCTL and HIGH ($P < 0.001$). Overtime (0–72 h), OM degradation of NCTL was the smallest ($P < 0.001$). There were no difference for DM, OM, and NDF degradation at 24, 48, or 72 h among feeds. Degradation of ADF at 24 h or 72 h did not differ among feeds but at 48 h showed difference between NCTL vs. other feeds with either binders ($P = 0.006$).

Key Words: feeding behavior, feed binder

Animal Health 1: Transition Cow

M30 Population approach for determining behavioral thresholds associated with health status during transition. D. Manriquez and P. Pinedo*, *Colorado State University*, *Fort Collins, CO.*

Monitoring of behavioral variables has been used to assess individual health and estrus presentation in dairy cows. The objective was to evaluate population dynamics of behavioral variables during postpartum to determine cut-off values associated with the subsequent occurrence of health events up to 21 DIM. Cows (n = 1,350) in an organic certified herd in CO, were affixed with CowManager tags (AABV, the Netherlands) for monitoring of rumination, eating, and active times (min/d). Health events were retrieved from on-farm software (PCDart, Raleigh, NC). Cows were categorized as healthy (HT) or diagnosed with at least one health event (DZ) within 21 DIM. Explanatory variables included behavioral variables summarized in 20 min intervals at 0, 1, and 2 DIM. Logistic regression and ROC curves analysis were used to calculate cut-off values maximizing sensitivity (Se, %) and specificity (Sp, %). Calving season, parity, and dystocia were included in the models as controlling variables when $P < 0.1$. Overall, 17.2% of the cows were in the DZ category. Increments of the behavioral variables were associated with lower odds of disease presentation. For each 20 min/d increment in rumination at 0, 1, 2 DIM the odds of DZ decreased by 0.95 (95% CI: 0.92–0.98), 0.92 (0.9–0.95), and 0.90 (0.87–0.92), respectively. For each 20 min/d increment in eating time at 0, 1, 2 DIM the odds of DZ decreased by 0.89 (0.86–0.92), 0.85 (0.82–0.89), and 0.84 (0.8–0.88), respectively. For active time, the odds of DZ decreased by 0.96 (0.93–0.99), 0.94 (0.91–0.96), 0.95 (0.92–0.98), respectively. Eating time at 1 DIM had the greatest area under curve (AUC = 0.69), followed by eating (AUC = 0.68) and rumination time (AU = 0.64) at 2 DIM. The cut-off values that optimized Se and Sp at these points were 233.5 (Se = 65; Sp = 61), 285.9 (Se = 63.2; Sp = 61.3), and 438 (Se = 61.3; Sp = 52) min/d, respectively. AUC for active time was < 0.6 and Se and Sp < 50%. This study suggests that rumination time and eating time have discriminative value at early lactation for cows at risk of health disorders and these assessment points could be used for developing targeted prophylaxis after calving.

Key Words: behavior, disease, performance

M31 Consistency of lying time is associated with reduced serum nonesterified fatty acids of prepartum dairy heifers and cows. B. T. Menichetti*[1], J. M. Piñeiro[2], A. Garcia-Guerra[3], A. E. Relling[4], W. P. Weiss[4], and G. M. Schuenemann[1], [1]*Department of Veterinary Preventive Medicine, The Ohio State University*, *Columbus, OH*, [2]*Department of Animal Science, Texas A&M AgriLife Extension Service*, *Amarillo, TX*, [3]*Department of Animal Sciences, The Ohio State University*, *Columbus, OH*, [4]*Department of Animal Sciences, The Ohio State University*, *Wooster, OH.*

The objective was to assess the association of pre-partum lying time (LT) with serum nonesterified fatty acids (NEFA) in prepartum dairy heifers and cows. A total of 723 Holstein pregnant dairy heifers (n = 229) and multiparous cows (n = 494) from 3 dairy herds were enrolled at 14 ± 3 d before parturition (dpp). A cohort of 20 to 36 cows was enrolled monthly at each dairy herd and electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual heifers and cows to assess their LT. To assess consistency of LT, the coefficient of variation (CV) of LT was computed for each individual animal by dividing the SD by mean LT within 7 d before blood NEFA collection and reported as an absolute ratio. Blood samples were collected from animals at 7 ± 3 dpp for serum NEFA concentration. Herd 1 regrouped animals 3 times per week while herds 2 and 3 regrouped animals once per week. PROC CORR procedure of SAS was used to assess the relationship between CV of LT and prepartum serum NEFA. Correlations were adjusted by parity, body condition score (BCS) at enrollment, season (winter, spring, summer and fall), and herd. Season, herd, parity, and BCS were associated with CV of LT ($P < 0.05$). Pregnant heifers had an overall mild (r = 0.28, $P < 0.0001$; herd 1: r = 0.13, herd 2: r = 0.52, and herd 3: r = 0.19) significant positive correlation between mean CV of LT and serum NEFA. Multiparous cows had an overall weak (r = 0.11, $P = 0.01$; herd 1: r = 0.07, herd 2: r = 0.13, and herd 3: r = 0.14) significant positive correlation between mean CV of LT and serum NEFA. The greater the CV of LT (<0.10 vs >0.30) within 7 d before blood collection, the greater the concentration of serum NEFA (302 µEq/L vs 450 µEq/L; $P = 0.005$). These findings provide evidence that consistency of LT of prepartum heifers and cows should be considered when troubleshooting metabolic problems at the herd level.

Key Words: prepartum, lying time, NEFA

M32 Metabolite health index relationship with metabolism and milk yield and its composition of postpartum dairy cows. E. J. Askel[1], M. Poczynek[1], A. M. Fillus[1], I. F. Carrari[1], J. H. Carneiro[1], J. C. S. Lourenço[1], G. F. M. Leão[2], and R. Almeida*[1], [1]*Universidade Federal do Paraná*, *Curitiba, PR, Brazil*, [2]*Castrolanda Cooperativa Agroindustrial*, *Castro, PR, Brazil.*

The goal of this study was to use the metabolite health index (MHI) categorization to evaluated metabolism and productive performance of Holstein postpartum cows. Four hundred 21 cows were evaluated, 173 primiparous and 248 multiparous, from 26 herds in Paraná State, Southern Brazil. Each cow had a single blood sample collected between 0 to 21 DIM. Serum AST, GGT, BHB, NEFA, Ca, glucose, albumin, bilirubin, and cholesterol were evaluated. A MHI was calculated using albumin, bilirubin and cholesterol concentrations, as well as their general means and SD (Gallagher et al., 2019) within 3 classes of lactation week; between 0 to 7 DIM, 8 to 14 DIM, and 15 to 21 DIM. All cows were categorized as medium (mean MHI ± 0.5 SD; n = 185 cows; MHI = 0.152), low (<mean MHI – 0.5 SD; n = 108 cows, MHI = −2.832) and high (>mean MHI + 0.5 SD; n = 128 cows; MHI = 2.17) groups, and each cow was categorized within its DIM range. Milk yield (kg), and fat and protein contents in the first test-day were recorded. Fat:protein ratio (FPR) was calculated and accumulated milk up to 30 and 100 DIM (kg) were recorded. Statistical analysis was conducted with GLM procedure from SAS. Cows in the low MHI group had higher BHB (1.00 vs. 0.64 mmol/L; $P < 0.01$), AST (104.3 vs. 84.3 U/L; $P < 0.01$), and NEFA (0.83 vs. 0.47 mmol/L; $P < 0.01$), and lower glucose (58.0 vs. 61.2 mg/dL; $P < 0.01$), and Ca (8.9 vs. 10.1 mg/dL; $P < 0.01$) than the high MHI group. It was observed higher ($P < 0.01$) milk yields at the first test-day for medium and high MHI groups than for low MHI group (39.1 and 37.9 vs. 35.2 kg/d, respectively). But there were no differences ($P > 0.05$) in accumulated milk up to 30 and 100 DIM, and milk fat content among MHI classes. It was observed lower ($P < 0.01$) milk protein content for low MHI group than for medium and high classes (3.10 vs. 3.21 and 3.26%), so these last 2 groups had lower FPR (1.18 and 1.19 vs. 1.26) than the high MHI. Our results suggest that there is an effect of the MHI class on milk yield in the first test-day, and there are effects on some blood metabolites which are not included in the MHI calculation.

Key Words: liver health, transition period

M33 Metabolic markers for purulent vaginal discharge and subclinical endometritis in dairy cows. O. Bogado Pascottini* and S. LeBlanc, *Ontario Veterinary College, University of Guelph*, *Guelph, ON, Canada.*

This study aimed to compare serum markers for systemic inflammation (SI), and liver and energy metabolism in samples obtained −7, 1, 3, 5, 7, 14, 21, and 35 d relative to calving from healthy dairy cows and those diagnosed with purulent vaginal discharge (PVD) or subclinical endometritis (SCE) at 35 d postpartum. Measured metabolites in serum were total calcium (tCa), total protein, albumin, globulin, cholesterol, urea, glucose, gamma-glutamyl transferase, aspartate aminotransferase, glutamate dehydrogenase, β-hydroxybutyrate (BHB), nonesterified fatty acids (NEFA), haptoglobin (Hp), and insulin-like growth factor-1 (IGF-1). Holstein cows were classified healthy (neither PVD nor SCE; n = 38), PVD (n = 10) or SCE (n = 10) at 35 d postpartum. The cut-point for PVD was mucopurulent vaginal discharge or worse, measured with Metricheck, and for SCE > 5% endometrial polymorphonuclear cells. PVD and SCE were mutually exclusive categories. The association of each blood serum metabolite with reproductive tract health classification was fitted in mixed linear regression models, accounting for repeated measures, sampling day, parity, BCS, and interactions of status and day. Serum haptoglobin was greater at 3, 5, 7, and 15 d postpartum for SCE and at 7 and 35 d postpartum for PVD in comparison to healthy cows. Albumin concentrations were lesser for PVD than healthy at 15 d postpartum and lesser for SCE than healthy at 35 d postpartum. The week before calving, SCE had lesser tCa than healthy cows, and at 7 and 15 d postpartum PVD had lower tCa than healthy cows. At 15 d postpartum, serum NEFA, BHB, and globulin were greater, and IGF-1 lower for SCE than PVD or healthy cows. For all other metabolites, no differences were found. Although PVD or SCE had more indication of postpartum SI (high Hp and low albumin) than healthy cows, markers of energy status were more compromised in SCE than in PVD or healthy cows. This supports the hypothesis that SCE is associated with maladaptation to postpartum metabolic demands and (sterile) SI rather than inflammation associated with uterine infection, as previously demonstrated by other authors for PVD.

Key Words: transition period, uterine disease, inflammation

M34 Late gestation conditions leading to postpartal subclinical ketosis in dairy cows affects offspring growth and performance.

N. A. Carpinelli*[1], J. Halfen[1,2], S. D. L. Ramirez[1,3], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University*, *Brookings, SD*, [2]*Núcleo de Ensino, Pesquisa e Extensão em Pecuaria (NUPEEC), Universidade Federal de Pelotas*, *Pelotas, RS, Brazil*, [3]*Universidad de la Salle*, *Bogotá, DC, Colombia*.

Ketosis is a common disease associated with NEB in peripartal dairy cows, and the onset of this condition is linked to prepartal preconditions such as decreased intake and excessive fat mobilization. Likely the same prepartal endocrine peripheral changes that predispose cows to subclinical ketosis (SK) postpartum will affect fetal development and colostrum biosynthesis. Thus, the objective of this retrospective study was to evaluate the effects of maternal SK in peripartal dairy cows on offspring growth and development. Twelve Holstein dairy cows (n = 6/group) were monitored during the peripartal period. Cows were housed in bedded pack pens and fed the same close-up diet (0.63 Mcal/kg DM and 12.3% CP). After parturition cows were moved to a freestall barn and fed a lactation diet (0.73 Mcal/kg DM and 15.6% CP). Precision Xtra was used to measure blood BHB at 1, 3, 5, 7, 9, and 11 d postpartum and cows were classified as subclinically-ketotic (KET; > 1.4 mmol/L) or non-ketotic (NONKET; < 1.4 mmol/L). Dairy calves were enrolled if calving difficult < 3, BW ≥ 32kg, single calf, colostrum quality ≥ 21% Brix refractometer, and 3.8 L of colostrum intake from the same dam. Calves born to dams in KET and NONKET groups were assigned accordingly. Calves were monitored from 1 to 8 wk of age. Calves were fed 2.8 L/d of milk replacer 2x/d from 1 to 6 wk, 1x/d in 7 wk, and weaned at 8 wk. Calves had ad libitum access to starter and water. The BW and withers height (WH) were measured weekly until wk 8. Health checks were performed daily. Data were analyzed using the MIXED procedure of SAS. Birth BW was greater ($P = 0.05$; 42.9 vs 37.9 kg) in KET calves than NONKET. However, a slower ($P = 0.02$) growth rate was observed in KET calves than NONKET from 1 to 8 wk (53.1 vs 56.2 kg). The latter was reflected in a lower ($P = 0.05$) final BW at 8 wk in KET than NONKET (71.3 vs 74.8 kg). Overall, offspring's WH was not affected ($P = 0.016$) by maternal SK. Results suggest that prepartal endocrine and metabolic mechanisms predisposing dairy cows to postpartal SK may produce long-lasting effects on the offspring's growth and development.

Key Words: calves, ketosis, fetal programing

M35 Effect of timing of prepartum vaccination relative to pen change of dairy cows on lying time and serum glucose, nonesterified fatty acids, and calcium at calving.

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The objective was to assess the effects of timing of prepartum vaccination (Enviracor J5 and Scourguard4; Zoetis) relative to pen movements of gestating dairy cows on lying time (LT), energy status (plasma glucose and nonesterified fatty acids [NEFA]) and plasma calcium at calving. Briefly, pregnant multiparous Holstein cows (n = 296) from one dairy herd were randomly allocated into 1 of 3 treatment groups at 35 ± 3 d before anticipated parturition (dpp): 1) vaccination at 28 d and cow pen change at 21 d (V28-M21; n = 104), 2) vaccination and cow pen change at 28 ± 3 d (V28-M28; n = 96) and 3) vaccination and cow pen change at 21 ± 3 d (V21-M21; n = 96). All prepartum cows were loosed-housed in barns with free access to a contiguous dry-lot patio. Every other week, a group of 43 to 53 animals were enrolled and electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual cows to assess their LT. Blood samples were collected at 28, 26, 21, 19, 14 dpp and at calving. Data were analyzed using MIXED procedure of SAS accounting for BCS, parity and cohort of enrollment. Regardless of treatment group, LT of prepartum cows ranged from 12 to 13.2 h/d. Cow movement with vaccination reduced LT ($P = 0.0003$) by 36 min/d for the first 3 d thereafter compared with cow movement alone. V21-M21 cows had higher concentrations of NEFA at 19 (170 μEq/L; $P < 0.0001$) and 14 dpp (161 μEq/L; $P = 0.01$) compared with V28-M28 (109 μEq/L and 130 μEq/L, respectively) or V28-M21 cows (102 μEq/L and 141 μEq/L, respectively) while no differences among groups were observed at calving. At calving, V28-M21 cows had higher ($P = 0.04$) glucose concentration (114.5 mg/dL) compared with V21-M21 cows (101.6 mg/dL) with V28-M28 cows intermediate to other 2 groups (107.2 mg/dL). V28-M21 cows had reduced proportion of hypocalcemia (≤8.0 mg/dL; 18.7%; $P = 0.004$) compared with V21-M21 (37.3%) but did not differ from V28-M28 cows (24.8%). These findings suggest that vaccinating cows at 28 dpp and pen change at 21 dpp would benefit common prepartum changes in metabolism.

Key Words: prepartum, fatty acids, hypocalcemia

M36 Blood biomarkers through an ex vivo LPS challenge under ketotic conditions in peripartal dairy cows.

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Negative energy balance is commonly experienced by dairy cows after calving and can lead to metabolic disorders such as ketosis, which can affect the performance and immunity of peripartal dairy cows. This study evaluated the effects of ketotic conditions in postpartal dairy cows on immunological parameters via an ex vivo LPS challenge and its relation to animal performance and blood biomarkers. Fourteen Holstein cows (n = 7/group) were monitored during the peripartal period. A retrospective analysis was performed by measuring blood BHB with the Precision Xtra at 1, 3, 5, 7, 9, and 11 DIM, and cows were classified as subclinically-ketotic (KET; > 1.4 mmol/L) or non-ketotic (NONKET; <1.4 mmol/L). Blood samples were collected at −30, −15, 5, 14, and 30 DIM to evaluate biomarkers of metabolism, inflammation, and oxidative stress. At 5 DIM, blood samples were collected for an ex vivo LPS challenge conducted at 0 (control), 0.01 (low dose), and 5 µg of LPS/mL (high dose) for 3.5h. After LPS challenge, the samples were centrifuged and plasma was obtained for biomarker analysis. The MIXED procedure of SAS was used to analyze the data. A Group × Day (G × D; $P < 0.01$) was observed in postpartal DMI, where lower DMI was observed in KET cows than NONKET during 1 and 2 wk postpartum. There was a trend ($P = 0.15$) for greater milk yield in NONKET cows than KET (37.3 vs 32.4 kg). A G × D ($P < 0.01$) was observed in NEFA, which resulted in a trend ($P \leq 0.10$) for greater NEFA in KET than NONKET at 14 and 30 DIM. There was a trend ($P = 0.06$) for G × D in haptoglobin, where greater ($P < 0.01$) haptoglobin was observed in KET than NONKET at 5 DIM. In the ex vivo LPS challenge, a trend ($P = 0.06$) for Group × LPS was observed in myeloperoxidase (MPO), where MPO tended ($P = 0.09$) to increase with greater concentrations of LPS in KET while no change ($P = 0.29$) was observed in NONKET. A Group × LPS ($P < 0.01$) was observed in IL-1β, where greater ($P = 0.05$) IL-1β was observed in KET than NONKET at high LPS. These results confirm that ketosis plays an important role in the effective immune response to additional diseases that peripartal dairy cows may endure soon after calving.

Key Words: ketosis, metabolism, lipopolysaccharide

M37 Assessment of the effects of intrauterine dextrose on clinical cure rate, body condition score, and concentration of β-hydroxybutyrate and haptoglobin in postpartum dairy cows diagnosed with clinical metritis.

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The objective of this study was to assess the effects of intrauterine dextrose (50%) infusion on clinical cure rate, body condition score (BCS), β-hydroxybutyrate (BHB) and systemic inflammation (haptoglobin; HP) in dairy cows diagnosed with clinical metritis (CM). Cows (n = 351) from a dairy farm located in Pennsylvania were screened at 7 ± 3 DIM using a Metricheck device to assess vaginal discharge. Cows that presented a fetid red-brownish watery vaginal discharge (n = 53) were classified as CM cows, blocked by parity and randomly assigned to 1 of 2 groups: 1) CONV (n = 27): 2 injectable (SC) administrations of ceftiofur (6.6 mg/Kg; Excede, Zoetis Inc.) 72 h apart; and 2) DEX (n = 26): 3 intrauterine infusions of dextrose (50%; 1 L/cow) 24 h apart. Furthermore, cows that presented a normal vaginal discharge at 7 ± 3 DIM (i.e., dense clear discharge; healthy group; HLT; n = 27) were randomly selected and matched by parity to CONV and DEX cows. Cows were re-screened at 14 ± 3 DIM and 21 ± 3 DIM to assess clinical cure rate. Body condition score was assessed and blood samples (i.e., BHB, HP) were collected at enrollment and at 14 ± 3 DIM and 21 ± 3 DIM. The data were analyzed using the MIXED and GLIMMIX procedures of SAS as a randomized complete block design. There was no difference in clinical cure rate at 14 ± 3 DIM (CONV = 50.27 ± 12.49%; DEX = 45.9 ± 12.37%) and 21 ± 3 DIM (CONV = 88.05 ± 6.59%; DEX = 88.51 ± 6.35%) between CONV and DEX groups. HLT cows tended to have a higher BCS at 21 ± 3 DIM compared with cows in the CONV and DEX groups (CONV = 3.05 ± 0.09 pts.; DEX = 2.96 ± 0.09 pts.; HLT = 3.25 ± 0.10 pts.). There was no difference in BHB concentrations between study groups. Overall, cows treated with DEX had higher HP concentrations compared with HLT cows (CONV = 119.91 ± 23.52 µg/mL; DEX = 153.06 ± 22.75 µg/mL; HLT = 71.52 ± 23.91 µg/mL). These results suggest that, although intrauterine infusion of dextrose may achieve similar clinical cure rates as a conventional antibiotic treatment, the treatment may not be as effective on decreasing systemic inflammation in CM cows.

Key Words: metritis, dextrose, inflammation

M38 The effect of vaccination with a *Mannheimia haemolytica* subunit vaccine on milk yield in lactating dairy cows.

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Many herds in the US use a *Mannheimia* vaccine in lactating dairy cows as an aid in reducing the impact of Bovine Respiratory Disease (BRD. A prospective, randomized clinical trial was conducted to evaluate milk production changes associated with the administration of a *Mannheimia haemolytica* subunit vaccine (MHSV; Nuplura PH, Elanco Animal Health, Greenfield, IN). A total of 972 lactating Holstein dairy cow were randomized into 1 of 3 study groups on a Midwestern dairy. Daily milk production during the 7 d before treatment was averaged and used as the baseline for comparison. Lactation Group (1, 2, or 3+), DIM, and 7-d pretreatment average milk production (41.7 kg) was not different across the groups. Cows were treated at the end of the morning milking on vaccination day according to group assignment of (MHSV), Saline, or Negative Control (no injection). Cow-level milk production was then averaged for vaccination day (Day 0) and the following 2 d. The 3-d post-treatment milk average was then compared with the baseline 7 d average milk yield for each group. The association between vaccination with MHSV and subsequent milk production changes while controlling for explanatory factors was compared with both the Saline and Negative Control groups using ANOVA and fitting least squares using JMP Pro 14.3.0. Negative Control cows served as the referent value. Saline group's decline in milk was 0.25 kg greater than Negative Control cows but the difference was not significant ($P = 0.57$). The Nuplura group's decline in milk was 0.7 kg more than the Negative Control, $P = 0.02$, but the Saline Nuplura declines were not significantly different ($P = 0.17$). On Day 1, the Nuplura group loss was 1.18 kg more than the Negative Control cows ($P < 0.01$) but the Saline group's loss of 0.13 kg was not different from Negative Control ($P = 0.92$). On Day 0 and Day 2, there were no significant differences between groups.

Key Words: *Mannheimia* vaccine, dairy cattle, bovine respiratory disease

M39 OmniGen-AF and OmniGen Pro improve immunocompetence of ewes subjected to dexamethasone-induced immunosuppression.

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OmniGen-AF (OGAF; Phibro Animal Health, Teaneck, NJ) is a feed additive with demonstrated benefit on improving cellular immunocompetence of stressed animals. OmniGen Pro (OGPRO, Phibro Animal Health) is a new product built on the OmniGen foundation, developed to maintain the principles of OGAF while improving gastrointestinal tract function and integrity. This study aimed to confirm the similar immunological effects of these 2 products. Fifteen Dorset ewes (39 kg BW) were randomly assigned to 1 of 3 diets: control (no additive), OGAF (6 g OGAF/head/d), and OGPRO (6.75 g OGPRO/head/d). Ewes were fed 227 g of grain, with the additives, and ad libitum hay for 23 d. On d 20, ewes were IV-injected with dexamethasone (DEX) at a rate of 0.4 mg/kg BW/day for 3 d. D 0, 20 and 23 blood draws were used to quantify whole blood gene expression of IL-8-Rβ and L-selectin (qPCR), isolated-neutrophil L-selectin protein abundance (Western blot) and neutrophil functional activity (phagocytosis and oxidative burst capacity, flow cytometry). Data were analyzed with 2 contrasts: Control vs. OmniGen (OGAF + OGPRO) and OGAF vs. OGPRO, and significance declared at $P \leq 0.05$. Feed intake and BW gain did not differ ($P > 0.05$) between treatment groups during the study. L-selectin gene expression was greater for OmniGen-fed ewes at d 20 ($P = 0.05$) and 23 ($P < 0.01$). Pre-DEX challenge, IL-8-Rβ gene expression, L-selectin protein abundance, and neutrophil functional activity did not differ ($P > 0.05$) between treatment groups. Following the DEX challenge, OmniGen increased IL-8-Rβ gene expression ($P = 0.02$), L-selectin protein abundance ($P = 0.03$), neutrophil phagocytosis ($P < 0.01$) and oxidative burst activity ($P < 0.01$). No differences were observed ($P > 0.05$) between OGAF and OGPRO for L-selectin (gene expression and protein abundance), IL-8-Rβ (gene expression), nor for neutrophil functional activity before or after

DEX. This study confirms OGAF and OGPRO have similar beneficial effects on preventing the immunosuppressive effects of dexamethasone.

Key Words: OmniGen, immunocompetence, immunosuppression

M40 Low expression of sirtuin 1 in the dairy cows with mild fatty liver alters hepatic lipid metabolism.

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This study aimed to investigate the effect of hepatic steatosis on mRNA and protein abundance of the sirtuin 1 (SIRT1), along with the target mRNA and protein expressions and activities of related to lipid metabolism in liver tissue. Lactating Holstein multiparous cows (days postpartum 70 ± 12) of the same breed, age and having similar milk production characteristics and body condition scores were selected from a commercial dairy farm. Control cows (n = 6, parity 3.0 ± 2.0, milk production 28 ± 7 kg/d) and mild fatty liver cows (n = 6, parity 2.3 ± 1.5, milk production 20 ± 6 kg/d) were retrospectively selected based on liver triglycerides (TG) content (% wet liver)(control = 0.45, fatty liver = 7.65, 5–10 was considered as mild fatty liver) . Dairy cows were slaughtered for collecting liver tissue samples and processed as part of the normal work of a commercial abattoir. One-way ANOVA (with Dunnett's post hoc test) and a 2-tailed Student's *t*-test were performed by Statistical Package for the Social Science (SPSS) 17.0. Compared with the control group, fatty liver cows had greater concentrations of cholesterol and TG along with the typically vacuolated appearance and greater lipid droplets in the liver. Furthermore, fatty liver cows had greater mRNA and protein abundance related to hepatic lipid synthesis (SREBP-1c, ACSL, ACC and FAS) and lipid transport (L-FABP, ApoE, LDLR and MTTP) ($P < 0.05$). However, they had lower mRNA and protein abundance associated with fatty acid β-oxidation (SIRT1, PGC-1α, PPARα, RXRα, ACO, CPT1, CPT2 and LCAD) ($P < 0.05$). Besides, mRNA abundance and enzyme activity of Cu/Zn SOD, CAT, GSH-Px and Mn-SOD decreased and mRNA and protein abundance of Nrf1 and TFAM decreased ($P < 0.05$). Lower enzyme activities of SIRT1, PGC-1α, Cu/Zn SOD, CAT, GSH-Px and Mn-SOD ($P < 0.05$) and concentration of GSH ($P < 0.05$) were observed in fatty liver cows. Overall, data suggest that low SIRT1 expression associated with hepatic steatosis promotes hepatic fatty acid synthesis and inhibits fatty acid β-oxidation. Hence, SIRT1 might be a novel therapeutic target for coping with fatty liver in dairy cows.

Key Words: dairy cow, SIRT1, fatty liver

M41 Validation of putative target genes of nuclear factor erythroid 2-related factor 2 (NRF2) in bovine.

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Finding ways to ameliorate the oxidative stress experienced by high-producing dairy cattle in the periparturient period is one of the many challenges faced by researchers and producers focusing on dairy production. The nuclear receptor transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) has been studied in non-ruminant models and may be utilized in ruminants to reduce oxidative stress through the upregulation of genes associated with antioxidant defense and cell survival. This is extremely important in the mammary gland where heightened oxidative stress can cause cell death and reduce milk production. Despite this, research on NRF2 in ruminants is limited and there is a need to identify the genes affected by NRF2 modulation and the associated biological outcomes. In this study, the aim was to validate in bovine a set of 7 putative NRF2 target genes as established in monogastric animals. This was achieved by using a combination of 10 μM of the NRF2 agonist sulforaphane (SFN), 100 nM of the NRF2 antagonist brusatol (BRU), NRF2-translation inhibition via Morpholino, and an untreated control in immortalized bovine mammary epithelial cells (MAC-T). RNA was extracted for RT-qPCR. Genes upregulated by SFN were GSR, KEAP1, NFE2L2, GCLC and NQO1 while treatment with BRU resulted in significant downregulation of GSTM1 and NQO1, but upregulation of GCLC and NFE2L2. Furthermore, treatment of cells with Morpholino resulted in downregulation of GSTM1, GPX1, and NQO1 but upregulation of GCR, GCLC, and KEAP1. Data indicated that transcription of GSTM1 and GPX1 requires a basic activation of NRF2 but is not induced by it. Transcription of GCLC and NFE2L2 is partly controlled by NRF2 but the regulation is complex, likely involving other transcription factors. The data revealed that NQO1 is the only transcript among the ones tested that is tightly regulated by NRF2 (i.e., true NRF2 target). All the other putative NRF2 targets are likely controlled by additional transcription factors in bovine mammary cells. Our data highlighted the problem of using in ruminant target genes previously identified in monogastric animals and, thus, the need to identify reliable NRF2 targets in bovine.

Key Words: NRF2, cow, RT-qPCR

M42 Whole-transcriptome analysis of nuclear factor erythroid 2-related factor 2 (NRF2) modulation in bovine mammary cells.

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The nuclear factor erythroid 2-related factor 2 (NRF2) has been well studied in non-ruminant models and has gained increased attention for its role as a potential target in promoting antioxidant defense and cell survival. The role of NRF2 in bovine and other ruminant models is not well studied. Given the extreme metabolic changes and associated oxidative stress encountered by dairy cattle as they transition from pregnancy to lactation, there is a clear need to better understand the role of NRF2 in ruminants. To accomplish this, MACT cells were treated with sulforaphane (SFN), a putative NRF2 activator, brusatol (BRU), a NRF2 inhibitor, and Morpholino (MOR), a synthetic oligonucleotide inhibitor of NRF2 translation. After 24h of treatment, both BRU and MOR decreased > 80% while SFN increased > 8-fold NRF2 activation as assessed via a gene reporter assay. RNA was extracted, prepared for sequencing using the QuantSeq kit (Lexogen), and sequenced using HighSeq 3500 (Illumina). The RNA-Seq data were normalized by RPM and statistically analyzed via ANOVA using JMP Genomics (SAS). Functional analysis of differentially expressed genes (DEG; FDR ≤ 0.05) compared with an untreated control was performed using DAVID and the Dynamic Impact Approach. The 943 DEG by BRU are associated with overall inhibition of metabolism, especially amino acid and energy metabolism. MOR had 421 DEG associated with activation of inflammatory and immune responses while inhibiting collagen and extracellular matrix. The 502 DEG by SFN are associated with induction of disulfide bonds, glycoproteins and selenoamino acid metabolism, but associated with inhibition of metabolism of other amino acids, ATPase activity, and intracellular trafficking. There were more DEG with opposite effects between BRU and SFN than MOR and SFN (151 vs. 48), indicating that BRU and SFN are modulating not only NRF2. Our study provided the first characterization of NRF2 role in bovine mammary cells highlighting a role of NRF2 that goes beyond the oxidative stress response.

Key Words: NRF2, cow, RNA-Seq

M140 Plasma alpha-1-acid glycoprotein concentration is associated with key blood biomarkers and disease incidence during the transition period.

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Alpha-1-acid glycoprotein (AGP) is an anti-inflammatory acute-phase protein which is negatively associated with feed intake in postpartum

dairy cows. Our objective was to determine associations between AGP and other biomarkers in transition dairy cows, and to evaluate the utility of AGP for predicting postpartum disease risk. Plasma samples (n = 2,086) from 434 Holstein cows in 6 studies were analyzed for AGP, glucose, insulin, free fatty acids (FFA), β-hydroxybutyrate (BHB), and haptoglobin on d −21, −12 ± 3, −3, 1, 3, 7 ± 1, 14 ± 1, and 21 ± 1 relative to parturition. A model was constructed with linear and quadratic fixed effects of AGP, with random effects of treatment(study). Quadratic effects with $P > 0.20$ were removed. Nominal logistics were used to determine the association of AGP concentration with disease incidence using fixed effects of AGP and study. On d −12, AGP was quadratically associated with BHB ($P = 0.02$) and negatively associated with insulin ($P = 0.04$), and d −3 AGP was positively associated with d 21 FFA ($P = 0.04$). On d 3 and 7, AGP was positively associated with BHB on d 7 ($P < 0.01$). Haptoglobin and AGP were positively associated on d 3 ($P < 0.001$), d 7 ($P < 0.001$), and quadratically associated on d 14 ($P = 0.02$). On d −12, AGP was associated with postpartum incidence of retained placenta (RP; $P = 0.05$) and tended to be associated with milk fever ($P = 0.06$). Plasma AGP on d 3, d 7, and d 14 was highly associated with postpartum incidence of displaced abomasum (DA), RP, and metritis ($P < 0.001$). Receiver operating characteristics for the association between AGP and postpartum DA incidence on d 3 ($P = 0.02$), d 7 ($P < 0.001$) and d 14 ($P = 0.05$) were excellent, with area under the curve of 0.99, 1.0, and 0.93, respectively. Plasma AGP on d 1 ($P = 0.06$) and d 3 ($P = 0.09$) tended to be associated with retention to 280 d in milk. Despite significant associations between AGP and postpartum disease, its utility for disease forecasting was less promising. Nevertheless, limited associations between AGP and metabolic biomarkers suggest it may serve as a unique predictive variable.

Key Words: acute-phase protein, biomarker

Dairy Foods 1

M43 Evaluation of the effects of gamma irradiation treatment on the compositional, textural, color, volatile profile, and microbiological quality of an artisanal hard-pressed cheese. F. Nyamakwere[1], G. Esposito[2,1], K. Dzama[1], P. Gouws[1], T. Rapisarda[3], G. Belvedere[3], and E. Raffrenato*[2,1], [1]*Stellenbosch University*, *Stellenbosch, South Africa*, [2]*RUM&N Consulting*, *Reggio Emilia, Italy*, [3]*Consorzio per la Ricerca nel settore della Filiera Lattiero-Casearia e dell'agroalimentare*, *Ragusa, Italy*.

Irradiation treatment can be an effective way of reducing the incidence of food-borne pathogens on cheese, thus improving food safety. The objective of this study was to evaluate the efficacy of γ-irradiation against *Listeria monocytogenes, Escherichia coli,* coliforms and aerobic colony counts (ACC). At the same time effects on cheese composition, texture, color and odor properties were evaluated. The cheeses were manufactured at 4 farms using raw milk under artisanal processing conditions and aged for 60 dd. Samples were of either 250 or 500 g. The Cobalt-60 γ-irradiator was used at a maximum dose of 5.0 kGy, dose rate of 1 kGy/h and source strength was 150 kCi. Cheese samples both before and after treatment were analyzed by the "SMart Nose" system, by gas chromatography-mass spectrometry-olfactometry. Data were analyzed with the irradiation treatment and sample weight as the main fixed factors. Moisture, pH, total nitrogen, fat in dry matter, water activity were reduced ($P < 0.05$) and salt and salt in moisture increased ($P < 0.05$) after the irradiation treatment. All values were within the acceptable range for hard cheese. Dose and treatment length altered water-holding capacity and some enzymatic and bacterial activities affecting the mentioned parameters. All color parameters (lightness, redness, yellowness, chroma and hue angle) were decreased ($P < 0.05$) by the irradiation treatment. Hardness and chewiness values increased ($P < 0.05$), whereas, cohesiveness and springiness decreased ($P < 0.05$). The amounts of β-casein decreased ($P < 0.05$) after the treatment. SMart Nose on the principal component analysis and Odor Active Compounds showed differences ($P < 0.05$) between the non-irradiated and irradiated samples. The irradiation treatment caused a significant ($P < 0.05$) reduction of *L. monocytogenes, E. coli,* coliforms and ACC on the treated cheese samples. Results and the low cost suggest the potential use of the irradiation treatment as an affordable method to effectively control food pathogens for resource limited producers.

Key Words: volatile profile, ionizing radiation, microbial safety

M44 The viability of probiotics during ripening and storage in Pladolens: A new Russian semi-hard cheese. O. Musina[1], E. Ott[1], J. Allen[2], and S. Roohinejad*[2], [1]*Siberian Research Institute of Cheese-Making*, *Federal Altai Scientific Centre of Agro-Bio Technologies, Barnaul, Russia*, [2]*Tillamook County Creamery Association*, *Tillamook, OR*.

Pladolens is a new Russian semi-hard cheese that is slightly sour and dense, elastic, homogeneous throughout the mass and has uneven holes with irregular slit-like shape. The mass fraction of moisture, salt, fat (dry matter) and pH are 44.0 ± 1.0%, 1.75 ± 0.25%, 45.0 ± 5.0%, and 5.3 ± 0.1, respectively. The aim of this study was to evaluate the viability of probiotics (lactobacillus and bifidobacteria) in Pladolens during ripening (1 mo, T = 10 ± 2°C, relative humidity 85–90%) and storage (3 mo, T = 3 ± 3°C, relative humidity 80–85%). The cheese samples were produced using starter cultures in combination with 2 mixtures of probiotics including mixture 1: *L. lactis* ssp. *lactis, L. lactis* ssp. *cremoris, L. lactis* ssp. *lactis* biovar. *diacetylactis* and *Leuc. lactis* or *Leuc. mesenteroides* ssp. *cremoris, L. plantarum*, *B. longum* and *B. bifidum*, and mixture 2: *L. lactis* ssp. *lactis, L. lactis* ssp. *cremoris, L. lactis* ssp. *lactis* biovar *diacetylactis* and *Leuc. lactis* or *Leuc. mesenteroides* ssp. *cremoris, L. plantarum*, and *B. adolescentis*. Lactobacilli and bifidobacteria were grown in Rogosa agar and TOS-MUP agar with subsequent incubation of 72 h, respectively. The smallest real difference (SRD) was used to determine the extent of measurement error with 8 replications. A large number of lactobacillus live cells were observed in the cheese samples inoculated with both types of cultures after 90 d. In the samples inoculated with the mixture 1, the number of bifidobacteria after the press and 90 d storage were 2.0×10^5 cfu/g and 8.9×10^4 cfu/g, respectively, while in the mixture 2, the number of bifidobacteria was in the range of 8.5×10^5 cfu/g after the press and 9.0×10^4 cfu/g after 90 d of storage. Although the survival rate of bifidobacteria in the cheese samples was higher than after the press, no significant differences were observed during ripening and storage (SRD <2.0, $P > 0.05$). The results demonstrated the high viability of lactobacillus and bifidobacteria in Pladolens during ripening and storage until the expiration date (3 mo).

Key Words: Pladolens, semi-hard cheese, probiotics

M45 Manufacture of imitation Mozzarella cheese without emulsifying salts using acid curd and micellar casein concentrate. A. R. A. Hammam* and L. E. Metzger, *Dairy and Food Science Department, South Dakota State University*, *Brookings, SD*.

Imitation Mozzarella type cheese (IMC) is a dairy, partial or non-dairy food based on the source of protein and fat used in the formulation. IMC has the same basic principles of manufacture as process cheese (PC) and it is prepared by blending dairy ingredients and non-dairy ingredients with the aid of heat, shear, and emulsifying salts to produce a homogeneous product. Emulsifying salts are critical for the functional characteristics of IMC because they improve the emulsification characteristic of casein by displacing the calcium phosphate complexes that are present in the insoluble calcium-paracaseinate phosphate network in the casein containing ingredients. The objective of this study was to manufacture IMC using a combination of acid curd (AC) and micellar casein concentrate (MCC) that would provide the required emulsion capacity without the use of emulsifying salts. The formulations were targeted to produce IMC with 18.0% protein, 49.0% moisture, 20.0% fat, and 1.5% salt. In the IMC formulation, the AC was blended with MCC so that the formula contained a 2:1 ratio of protein from AC relative to MCC. Additional dairy and non-dairy ingredients (milk permeate, vegetable oil, and salt) were also utilized in the formulations. The IMC was prepared by mixing all ingredients in a kitchen aid to produce a homogeneous paste. Approximately 25 g of the mixture was cooked in the rapid visco analyzer (RVA) for 3 min at 95°C with a 1000 rpm stirring speed during the first 2 min and 160 rpm during the last min. The cooked IMC was then transferred into molds and refrigerated until further analysis. This trial was repeated 3 times using 3 different batches of AC. No significant differences ($P > 0.05$) were detected in the cooked viscosity (7500 cP), hardness (95.0 g), melting temperature (50.0°C), melting diameter (31.5 mm), and stretchability (12.3 cm) of IMC made from different AC and was similar to typical IMC produced with emulsifying salts. We conclude that IMC can be made with no emulsifying salts when the formulation utilizes a 2:1 ratio of protein from AC relative to MCC.

Key Words: micellar casein concentrate, acid curd, imitation mozzarella cheese

M46 Liquid chromatography-tandem mass spectrometry analysis of glycomacropeptide from whey protein isolate. Y. Qu*, B. J. Kim, and D. Dallas, *Oregon State University*, *Corvallis, OR*.

κ-Casein glycomacropeptide (GMP), a 64-amino-acid peptide, is released from κ-casein after rennet treatment and is one of the major peptides in whey protein isolate (WPI). GMP has anti-inflammatory and antibacterial actions. GMP has 2 major amino acid sequences and many different modifications including glycosylation, phosphorylation and oxidation, yet no previous work has provided a comprehensive profile of all the different distinct GMP forms present in whey. The full characterization of the composition and structure of GMP is important to help to understand the bioactivity of GMP. We therefore aimed to develop an analytical method to profile GMP and GMP peptide fragments in WPI using Orbitrap mass spectrometry combined with a nano-liquid chromatography (LC). A commercial WPI was dissolved in water and purified by C18-solid-phase extraction and characterized by a nano-LC/Orbitrap MS/MS under electron-transfer/higher-energy collision dissociation mode. MS and MS/MS results were interpreted using Byos (Byonic and Byologic) processing and manual spectral inspection to verify structures. Forty-five distinct intact GMP forms were identified in the WPI. One intact GMP was without any modification, 5 intact GMP forms were glycosylated only, 4 were phosphorylated only, one was oxidized only, 3 were both phosphorylated and oxidized, 22 were for both glycosylated and phosphorylated and 8 were glycosylated, phosphorylated and oxidized. Four O-linked glycosylations (HexNAc1Hex1, HexNAc1Hex1NeuAc1, HexNAc1Hex1NeuAc1, HexNAc1Hex1NeuAc2) were present on the GMP. In addition to intact GMP, 186 distinct GMP-derived peptides were identified in the WPI, likely generated from partial hydrolysis during whey processing or storage. These glycopeptides were between 9 and 63 amino acid length. We have demonstrated the efficacy of this novel analytical approach to comprehensively profile the range of GMP and GMP-derived structures in whey protein isolates. Our comprehensive profile of all the different distinct GMP forms present in whey provides some fundamental information on determining how GMP is digested in human and understanding the bioactivity of GMP.

Key Words: glycomacropeptide, LC-MS/MS, whey

M47 Effect of inulin on the microbiological and organoleptic characteristics of synbiotic yogurt.

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Nowadays, there is an interest in adding probiotics and probiotics (synbiotic) to yogurt due to its health benefits. The objective of this work was to study the effect of different concentrations of inulin (0.2, 0.4, and 0.6%) on microbial and chemical characteristics of probiotic yogurt. The yogurt was manufactured with *Lactobacillus delbruckii* ssp. *bulgaricus* (Lb), *Streptococcus thermophilus* (St), and *Bifidobacterium bifidum* (Bb). Raw milk was received, pasteurized, and divided into 4 aliquots portions. All portions were inoculated with 1% Lb, 1% St, and 15% Bb. The first portion was utilized as control (T1) while 0.2, 0.4, and 0.6% of inulin were added to the second (T2), third (T3), and fourth (T4) portions, respectively. All treatments were inoculated at 40°C until a pH of 4.6 was reached. Subsequently, the yogurt was cooled and stored at 4°C for 16 d. Titratable acidity, sensory evaluation, Bb count, and total bacterial count (TBC) were determined during the storage. This experiment was repeated 3 times using 3 different batches of raw milk. The results showed that the addition of inulin has no significant effect ($P > 0.05$) on the titratable acidity of the yogurt during the 16 d of storage. There were no significant differences ($P > 0.05$) in the sensory evaluation of T1, T2, T3, and T4. The TBC increased ($P < 0.05$) over time in T1, while it was decreased ($P < 0.05$) with increasing the concentration of inulin in T2, T3, and T4. However, the addition of inulin increased ($P < 0.05$) the viability of *Bifidobacterium bifidum* during the storage. We conclude that inulin can be utilized in the manufacturing of synbiotic yogurt by incorporation with probiotic, which, in turn, enhances the growth of *Bifidobacterium bifidum* and antimicrobial activity that decreased the TBC. The impact of inulin in the texture of probiotic yogurt during 16 d of storage will be evaluated in subsequent studies by determining the rheological characteristics.

Key Words: probiotic yogurt, inulin, *Bifidobacterium bifidum*

M48 Microbial degradation of FD&C Red No. 40 in strawberry-flavored milk.

C. Rush* and J. Waite-Cusic, *Oregon State University, Corvallis, OR.*

Most high-temperature short-time (HTST) fluid milk processors declare their products are consumable 3 to 5 d after code date with minimal changes occurring in flavor and color. Flavored milks tend to display more significant quality defects near the end of shelf life which can negatively impact future purchase decisions. Most strawberry milk products include color additives, including FD&C Red No. 40 and/or red beet juice concentrate. The objective of this study was to observe the color degradation over shelf life in HTST strawberry-flavored milks dyed with FD&C Red No. 40 and to investigate a microbial cause for the color loss. Commercially available strawberry-flavored milks with FD&C Red No. 40 listed as a colorant (n = 2 brands, 3 separate lots) were obtained from local markets. At the code date, the milk was aseptically aliquoted into 50-mL conical tubes and stored at 7°C. Samples were analyzed daily for changes in pH, color, texture, and organoleptic properties (aroma). Once a defect was detected, the sample was spread plated on tryptic soy agar (TSA) and strawberry milk agar (standard methods agar with 10% vol/vol strawberry milk). Visual color degradation began one day post code date (2–4 log cfu/mL) displayed via white streaks on the surface of the sample. Seven days post code date (4–6 log cfu/mL) the samples with only FD&C Red No. 40 as the color agent were visually absent of the pink color (vibrant pink to opaque white) and samples with a secondary color agent had considerably reduced in color (vibrant pink to very light pink). Colonies were streaked on strawberry milk agar that contained FD&C Red No. 40 to select the color-degrading isolates. These isolates will undergo further sequencing to identify the species responsible for the degradation of FD&C Red No. 40 in strawberry-flavored milk.

Key Words: strawberry milk, FD&C Red No. 40, shelf-life

M49 Production and physico-chemical characterization of functional ice cream with whey and buttermilk powder.

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Consumers have sought healthier foods, with less chemical substances, fat, and sugar content. Natural ingredients are attractive for ice cream production, especially when it reduces thickeners and stabilizers. The use of dairy coproducts, such as whey and buttermilk, as ingredients in the manufacture of food products, is already a worldwide reality. Its use on dairy foods giving a sustainable destination to these coproducts, valuing the environmental appeal. For ice cream production, whey and buttermilk powder can be used due to their high protein content, excellent technological and nutritional characteristics. This work aimed to develop and characterize ice creams produced with whey and buttermilk powder. Formulations were produced with concentrations of whey and buttermilk between 5 and 15%, concentration of milk/cream between 70 and 90%, 10% of sugar, and 1% of emulsifier. The ice cream mix was pasteurized for 70°C for 30 min, and then churn it with the ice cream machine for 80 min. After that, the ice creams were stored at −18°C. Physico-chemical characterization was done by official methods, and results were evaluated by ANOVA and Tukey test ($P < 0.05$) for mean comparison. They presented pH between 6.24 and 6.50, acidity between 0.19 and 0.47 g acid lactic/100 g, moisture between 53.67 and 67.93%, ash between 0.92 and 1.69%; lipids between 8.94 and 9.89%, and lactose content between 7.85 and 18.09%. The results of colorimetry were lightness (L*) between 75.63 and 79.53, a* parameter between −0.80 and −1.73, b* parameter between +10.65 and +14.83. The formulation with higher contents of whey and buttermilk presented higher acidity, ash, lactose content, and trend to yellow color (+b*). The ice cream with a lower concentration of whey and buttermilk presented higher pH, moisture, lipids, lightness, and trend to green color (−a*). Ice cream production using whey and buttermilk is an innovative and viable alternative for the dairy industry, being able to bring technological benefits to the final product and benefits to the consumer's health.

Key Words: dairy products, functionality, innovative product

M50 Influence of protein content on acidity of fermented dairy beverages with buttermilk and gabiroba pulp (*Campomanesia xanthocarpa*).

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Milk is extremely nutritious and a complete food. The proteins, especially caseins, are one of the most important constituents to technological aspects and can directly influence the physical, chemical and nutritional characteristics of fermented products. The aim of this work was to correlate the influence of protein content with variation in acidity of fermented dairy beverages with buttermilk and gabiroba pulp (*Campomanesia xanthocarpa*). They were formulated by designing simplex centroid mixtures with different levels of milk (40–56%), whey (0–44%), buttermilk (0–44%) and gabiroba pulp (10- 20%). The beverages were analyzed for acidity, proteins and protein fractions, after manufactured after that weekly for 84 d, using official methods. The results were calculated statistically by ANOVA and the media were compared using the Scott-Knott test ($P < 0.05$). The acidity of the formulations on the first day presented results between 0.34 to 0.56 (g / lactic / 100mL) and that beverages with a higher protein content (2.12 to 2.24%) and caseins (1.69 to 1.88%) had a higher concentration of milk and varied values of whey, milk and pulp in their composition, indicating that milk was the ingredient that determined the highest acidity and protein content. The interactions of mathematical models confirm this result through the prediction equations that demonstrated that the interactions of ingredients with milk were antagonistic, that is, they not influence the increase in acidity. The increase in acidity over 84 d (increasing from 0.46 to 0.56 g lactic acid / 100mL) demonstrates an occurrence of post-acidification. However, as suggested minor changes in the acidity part of the beverages due to the natural acidity of milk, attributed to caseins, despite being an acidic component, it acts as a buffering agent, controlling post-acidification. Thus, the higher milk concentration of the formulations determined high acidity values, however the proposed values remain the minimum levels due to the buffering effect of caseins.

Key Words: caseins, cerrado fruit, functional

M51 Comparing some genetic determinants associated with colonization of *Listeria* isolates within dairy plant environment.

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Listeria monocytogenes is a high-risk pathogen that can colonize and persist in dairy processing plants by forming resilient biofilms. While some strains are repeatedly isolated from a plant environment, others appear to be merely transitory. The current study investigates the genetic determinants that could be associated with the biofilm-forming ability of some environmental *Listeria* isolates. Three isolates from the dairy processing environment, *L. monocytogenes, L innocua, and L. welshimeri* were compared for biofilm-forming ability on dairy floors. Small sterile chips (1 × 1 cm^2) of dairy bricks were submerged in sterile dairy effluents and held at 22.5°C for 48 h to form biofilms. Swab samples drawn at the end of incubation were plated on brain heart infusion agar to enumerate biofilm-embedded cells. Means were compared using ANOVA. Our results indicate variability in the biofilm-forming ability of the 3 isolates; *L. monocytogenes* (log cfu 2.50 ± 0.14/cm^2), *L. innocua* (log cfu 2.72 ± 0.26 /cm^2), *and L. welshimeri* (log cfu 3.32 ± 0.18/cm^2). The 3 *Listeria* species were characterized using genetic determinants influencing colonization and biofilm formation. Genomes were assembled using CLC Genomics Workbench; resulting assemblies were used to identify MSLT types based on the *Listeria monocytogenes* type profile from PubMLST (pubmlst.org). Mapping each of the isolates to the *Listeria monocytogenes* EGD-e reference genome discovered 110,342 high-quality single nucleotide variants (hqSNVs). The presence of genes related to capsular glycan, cell wall/ capsular LTP, biotin biosynthesis, and carbohydrate metabolism associated with amino sugars such as chitin were correlated with the biofilm formation. Further studies in this regard would help us identify the genes associated with colonization, and serve as potential targets for novel approaches such as autoinduction interrupters for preventing or limiting *Listeria* adhesion.

Key Words: whole-genome sequencing, biofilm, *Listeria*

M52 Fermentation kinetics of dairy-relevant sugars in acid whey conditions by *Saccharomyces*, *Kluyveromyces*, and *Brettanomyces* species.

V. K. Rivera Flores*, T. A. DeMarsh, and S. D. Alcaine, *Cornell University*, *Ithaca, NY*.

Acid whey (AW) from Greek yogurt is an underutilized by-product and a challenge for the dairy industry. One valued-added scheme is the fermentation of AW -with or without the addition of lactase- by yeast such as *Saccharomyces, Brettanomyces* and *Kluyveromyces* spp. to produce new styles of fermented beverages. Previous research in our group observed changes in fermentation performance with lactase addition. Therefore, this work aims to describe the fermentation kinetics of dairy-relevant sugars by *S. cerevisiae*, *K. marxianus* and *B. claussenii* in simulated AW conditions to study alcoholic fermentation as an alternative for AW's reinsertion into the food supply chain. For this purpose, 4 preparations of yeast nitrogen base with amino acids with 40 g/L of lactose (LAC), glucose (GLU), galactose (GAL) or a 1:1 mixture of glucose and galactose (GLU+GAL), with a final pH of 4.20, were used as fermentation media. Each medium was inoculated with *S. cerevisiae*, *K. marxianus* or *B. claussenii* to achieve an initial concentration of 4 × 10^6 cfu/mL in 500 mL and incubated at 25°C under anaerobic conditions, while density, pH, cell count, ethanol and organic acids were monitored. Statistical analysis was done using Tukey's HSD test. Results indicated that *K. marxianus* had a similar or better performance compared with *S. cerevisiae*, whereas *B. claussenii* sugar consumption rate was substantially lower. In particular, densities showed that there is no significant difference in the time that *K. marxianus* and *S. cerevisiae* need for the complete depletion of GLU (2 d, $P > 0.05$) and GAL (3 d, $P > 0.05$), and that GLU+GAL consumption was significantly faster in the presence of *K. marxianus* (3 d, $P < 0.05$). On the other hand, *B. claussenii* exhibited significant differences in sugar utilization while in LAC vs GLU+GAL, not completing the fermentation for the latter ($P < 0.05$); a phenomenon that will be explored further. These results provide a deeper understanding of dairy sugar utilization by relevant yeast, allowing for future work to optimize fermentations to improve valued-added beverage and ingredient production from AW.

Key Words: acid whey, fermentation, ethanol

M53 Preliminary studies on the use of fluorescence spectroscopy and chemometrics for classification of nonfat dry milk based on spore counts.

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Nonfat dry milk (NDM) is a popular ingredient in a wide range of shelf-stable food products. However, high spore containing NDM can lead to ropiness and introduce unwanted lipase and protease activity. The thermophilic and mesophilic spores can enter into raw milk through the cow, feed, and beddings at the farm level. Further, spore counts can increase during the manufacture of NDM due to the concentration factor as well as contamination from the matured biofilms formed on the equipment. Therefore, the spore count is a critical quality indicator to be monitored during production. Previous research suggests that dipicolinic acid (DPA) is present in the core of endospore and can be used as a fluorophore of interest for rapid detection of spores. This objective of this study was to use DPA fluorescence spectra and chemometrics to develop classification models based on the spore levels. Commercial NDM samples (n = 40) were procured within the United States. The reference spore counts (cfu/g of NDM) were obtained by heating reconstituted NDM (10%) at 100°C for 30 min, plated on Tryptic Soy Agar, and incubated at 55°C for 48 h. To release all available DPA and to remove interferents, the reconstituted NDM (10%) was autoclaved at 121°C for 30 min followed by acidification and centrifugation. The terbium chloride was added to the supernatant buffered to pH 5.6 to enhance the DPA fluorescence signal. Emission spectra of terbium DPA complex were collected between 450 and 650 nm fixed at the excitation of 270 nm. Classification models were developed using partial least square quadratic discriminant analysis (PLS-QDA), forward selection quadratic discriminant analysis (FS-QDA), and random forest (RF). It was found that random forest provided the highest mean classification accuracy of 87% while FS-QDA and PLS-QDA showed the mean accuracy at 84% and 83%, respectively (validated using bootstrapping technique). The results suggest the potential of using fluorescence spectroscopy to classify the NDM based on spore counts.

Key Words: *Bacillus* endospores, classification models, dipicolinic acid

M54 Low-level microbial contaminants in whey multiply rapidly on food contact surfaces under production conditions.

B. Selover* and J. Waite-Cusic, *Oregon State University*, *Corvallis, OR*.

As the time between sanitation events increases, bacteria can attach and grow on equipment and surfaces potentially developing biofilms that could impact dairy product quality. Prudent sanitation schedules should be implemented to mitigate biofilm development; however, scientific evidence is lacking to inform these decisions. The purpose of this study was to demonstrate the potential for naturally low levels of non-starter bacteria in Cheddar cheese whey to attach and develop biofilms on representative surfaces within the length of a typical production day (18 h). Whey was collected after cutting Cheddar cheese curds during normal production activities at the Oregon State University Creamery. Whey was rapidly cooled and held at 4°C until use. The whey was preheated to 35°C and pumped (1.1 L/h) through a lab-scale CDC bioreactor containing polypropylene and stainless steel coupons. Bulk whey from the bioreactor was sampled at 0, 12, 15, and 18 h and enumerated for starter lactic acid bacteria, coliforms, *Acinetobacter* and *Pseudomonas*. Coupons of each material were removed at 12, 15, and 18 h and analyzed for bacterial attachment and growth using standard enumeration methods and scanning electron microscopy (SEM). The experiment was replicated 3 times. Non-starter bacteria increased in whey from 1.8 Log cfu/mL to 6.4 Log cfu/mL in 18 h, while starter bacteria remained constant at 7.6 Log cfu/mL. After 18 h, coliform levels on coupons increased to 6.4 Log cfu/cm^2, whereas *Acinetobacter* and *Pseudomonas* counts each increased to 4.9 Log cfu/cm^2. Whey pH was effectively maintained at 5.9–6.4 throughout the experiment. Bacterial attachment occurred at about the same rate on both materials after 18 h. SEM showed even distribution of attached bacteria on stainless steel, whereas polypropylene harbored biofilms only in manufacturing defects (cracks, crevasses). These results demonstrate that naturally low levels of bacterial contamination in whey can lead to significant bacterial growth on manufacturing surfaces within an 18 h production shift. These findings can inform sanitation schedules for cheese and other dairy manufacturers.

Key Words: biofilm, whey, microbiology

M55 The effect of whey protein hydrolysate as a binder on the physical characteristics of agglomerated whey protein isolate.

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Wet agglomeration involves spraying a liquid binder on the powder in a fluidized bed chamber causing adhesion of wet particles due to viscous bridges between the particles. These bridges are then consolidated by the continuous supply of hot air to form agglomerated particles. The agglomerates have a porous structure and consequently improve dissolution rate and decrease apparent bulk density. The objective of this study was to evaluate the effect of whey protein hydrolysate (WPH) as a liquid binder on the physical properties of the agglomerated whey protein isolate (WPI). Three lots of WPH were obtained from a commercial manufacturer. A Top-Spray fluid bed granulator (Midi-Glatt, Germany) was used. The experiment was conducted in triplicate based on a 3 × 3 × 2 factorial design with pre-wet mass (60, 100, and 140 g), WPH concentration (15, 20, and 25%), and flow rate (11 and 16 rpm). The nozzle pressure, fluid bed pressure and fluid bed temperature were set at 0.65 bar, 0.45 bar, and 60°C, respectively. Agglomerated WPI samples were stored at 25°C and analyzed for particle size and shape, bulk density, and tapped density. The size and shape characteristics of agglomerates were evaluated using Morphology G3-ID (Malvern Instruments Ltd., UK). The mean circle equivalent diameter (CED), circularity, elongation, and convexity were 15.18 µm, 0.74, 0.273 and 0.95, respectively. No significant differences were observed for the CED and convexity ($P > 0.05$) for the main effects. The WPH concentration, pre-wet, and flow rate significantly ($P < 0.05$) influenced the elongation of the WPI agglomerates. Bulk densities of agglomerates were between 0.22 and 0.31 g/cm^3. Pre-wet mass significantly ($P < 0.05$) influenced the bulk density of the particles. This might be due to the differences in the formation and breakage of the agglomerates. Tapped densities for the agglomerated samples were between 0.29 and 0.40 g/cm^3 and no significant difference was observed ($P > 0.05$) between the main effects. Overall, pre-wet mass had the major effect on the agglomerates physical properties followed by the flow rate and the WPH concentration.

Key Words: agglomeration, whey, physical characteristics

Growth and Development 1

M56 Effects of a natural herbal extract on colostrum apparent efficiency of absorption and vigor of neonatal dairy calves. M. E. Reis*[2,1], M. C. Cantor[1], C. M. M. Bittar[2], and J. H. C. Costa[1], [1]*University of Kentucky*, *Lexington, KY*, [2]*University of São Paulo*, *Piracicaba, SP, Brazil*.

Calves can experience poor vigor after birth. Therefore, it is of interest to determine if vigor can be improved by providing a natural herbal extract (extract). The study aimed to evaluate the effect of a natural herbal extract on apparent colostrum absorption efficiency and vigor in neonatal dairy calves. Holstein dairy calves (n = 22) were randomly enrolled to receive either a negative control (15 mL of distilled water) or one 15-mL oral dose of extract (CalfPerk, TechMix, Stewart, MN, USA) 3 h after birth. Cows were watched on live camera to determine delivery time, dystocia score and to ensure no colostrum was sucked from the dam. At 1.5 h after birth, a total vigor score was assigned, combining heart rate, oxygenation rate, response to nasal stimuli and suckle reflex. Afterward, calves were removed from maternity pen, weighed, and placed into an individual pen (3 × 3 m). Blood samples were collected at 2.5, 6, 12, 24, 48, and 72 h and additional vigor scores were collected at 2.5 h, 3.75 h, before tube feeding colostrum replacer (Premolac Plus IgG, Zinpro, Chilton, WI) to calves at 4 h after birth. Calves were vigor scored once daily until 4 d of age. Baseline blood BRIX and vigor at 1.5 h before colostrum were not different between treatments; [placebo 7.32 ± 0.38% BRIX and extract 7.32 ± 0.40% BRIX (mean ± sd); $P > 0.10$]. Total vigor score 15 min before colostrum replacer was not different ($P > 0.10$) between treatments with a mean for placebo of 4.18 ± 1.40 and for extract of 4.18 ± 1.47. Linear models evaluated the effect of the extract on BRIX with time as a repeated measure, calf as subject, and birthdate as random effect. A logistic model evaluated the odds of extract improving a calf's vigor score from 1 to 3 d of life (vigor score was categorized as 0–3 poor, 4–5 average, and 6–8 strong). The natural extract was not associated with a change in BRIX (placebo 7.77 ± 0.10%, extract 7.72 ± 0.10% BRIX; LSM ± SEM). Similarly, there was no significant effect of extract on improving vigor likelihood (OR 1.73: 95% CI: 0.66–4.60; $P = 0.27$). Preliminary results suggest the extract does not affect passive transfer BRIX. Future research in this study will examine the herbal extract's impact on behavior, and vitality in young calves.

Key Words: dystocia, supplement, vitality

M57 Weekly body weight change in wild-type and slick-haired Puerto Rican Holstein calves during the first eight weeks of life. I. Colón-Rodríguez*, K. I. Domenech-Pérez, and H. L. Sánchez-Rodríguez, *University of Puerto Rico, Mayagüez Campus*, *Mayagüez, Puerto Rico*.

Previous studies have reported different growth trends and mature body dimensions between wild-type (WT) and slick-haired (SL) Puerto Rican Holsteins heifers and cows, respectively. However, these comparisons were carried out with animals older than 3 mo of age and, to the authors' knowledge, no data exists comparing younger animals. Thus, the present study aimed to compare the weekly BW recordings between WT (n = 10) and SL (n = 10) Puerto Rican female Holstein calves at the Agricultural Experiment Station in Lajas, Puerto Rico. From August 30 to November 28, 2019 20 female calves were recruited at birth in WT/SL pairs. Sires were evenly distributed between hair coat groups. The BW of each calf was recorded weekly, every Thursday afternoon during the first 8 weeks of life. During the first sampling the WT and SL groups were on average 4.75 ± 1.39 and 4.56 ± 2.19 d old, respectively. Calves were weaned at 45 d of age and maintained in the same individual cages until the end of the study. Data were analyzed by the GLIMMIX and REG procedures of SAS. There was no interaction between hair coat type and age affecting the calves' BW ($P = 0.6867$). No differences ($P = 0.2831$) were observed for BW between WT and SL calves with 44.58 ± 8.15 and 45.58 ± 8.35kg on average, respectively. However, age affected both groups BW ($P < 0.0001$). From 1 to 8 weeks of age BW increased at a linear rate in both the WT (BW = 3.31age + 29.02; $R^2 = 0.84$; $P < 0.0001$) and the SL calves (BW = 3.32age + 30.45; $R^2 = 0.83$; $P < 0.0001$). In the present study no differences in growth rates were observed between hair coat groups during the first 8 weeks of life. Future studies should be directed at evaluating the complete growth curve for both phenotypes.

Key Words: dairy calves, slick-haired Holstein calves, body weight

M58 The impact of early life events on future reproductive and milk production performance in Holstein dairy heifers. T. S. Steckler* and J. P. Boerman, *Purdue University Department of Animal Sciences*, *West Lafayette, IN*.

The objective of this study was to evaluate the long-term effects of pre-weaning events on heifer conception rate and 1st lactation milk production of calves raised in automatic calf feeders. Daily milk consumption (MC), 400 d predicted BW, average daily gain (ADG), heifer conception rate (CR), and 280 d 1st lactation milk production (280M) were collected between Oct. 1, 2015 to Jan. 31, 2020. Calves (n = 9,099) were fed milk replacer through an automated calf feeding system (feeders = 8) for 60 d (range: 48 – 126d), and fed whole pasteurized nonsalable milk of 5% F and 30% P enhancer added at 20 g per L of milk. Calves were weighed at birth and several other time periods before calving. Daily BW predictions were calculated for individual animals using 3rd order orthogonal polynomials and 400 d (421.0 ± 34.1 kg; mean ± SD) weights were used for this model. ADG was calculated based on predicted BW between 0 and 60 d and 0–400 d. Cumulative 60 d MC was 508.1 ± 67.3 L; range of 179.9–785.1 L. Average age at conception was 437.5 ± 45.0 d; range of 308 to 631 d (n = 5,193), and average 280M was 9,305 ± 1,371.8 kg; range of 712–13,358 kg (n = 2,508). ADG from 0 to 400 d was weakly negatively correlated with conception age (r = - 0.18; $P < 0.0001$); calves with higher ADG conceived earlier than calves with lower ADG. Cumulative 60 d MC was weakly negatively correlated with conception age (r = −0.10; $P < 0.0001$), with a 156 L reduction in MC pre-weaning leading to an 11.6 d decrease in heifer CR. Pre-weaning ADG (0–60 d) was weakly positively correlated with 280M (r = 0.08; $P < 0.0001$). Calves with a 60 d ADG of 0.90 ± 0.07 kg (bottom 25%) produced on average 234 kg less milk than calves with an ADG of 1.20 ± 0.06 kg (top 25%). Genetic milk also influenced 280M ($R^2 = 0.24$, $P < 0.0001$). There was an 1,853.4 kg difference between calves in the top and bottom 25% of genomic milk index. Heifers with higher pre-weaning ADG, increased milk consumption, and higher genomic milk indexes have reproductive and milk production advantages through their 1st lactation.

Key Words: pre-weaning, reproduction, milk production

M59 Evaluating physically effective fiber from hay in the diet of 2- to 4-month-old Holstein calves. K. Aragona*, T. Dennis, F. Suarez, T. Hill, and J. Quigley, *Provimi North America*, *Brookville, OH*.

Previous research from our group indicates during the weaning transition, fiber is poorly digested. However, we have also observed limited hay inclusion optimized post-weaning growth of calves. Little is understood about the role roughage particle size plays in the diet. The objective was to evaluate the effects of hay particle size on growth, intake and digestibility in weaned calves from 2 - 4 mo of age. Male Holstein calves (n = 98; 78 ± 9.1 kg initial BW; 2 blocks of calves) were housed in groups (4 - 5 steers/pen, 6 pens/treatment). The trial was conducted December to April. All treatments were fed the same textured calf starter (CS) that contained 37% whole corn, 35% protein supplement pellet, 25% whole oats and 3% liquid molasses (as-fed basis basis) blended with: 1) no hay (NOH); 2) 5% pelleted hay (PH); 3) 5% short (≤2.5 cm) particle length hay (SH) or 4) 5% long (~12.5 cm) particle length hay (LH). Pen was the experimental

Table 1 (Abstr. M60). Growth and intake during wk 7 (step-down weaning) and 8 and rumen measurements at dissection

	Treatment				
Item	LL	LH	HL	HH	SEM
ADG, kg/d					
Wk 7	0.13^{ab}	0.32^{a}	-0.06^{ab}	-0.27^{b}	0.11
Wk 8	0.97^{a}	0.64^{ab}	0.37^{ab}	0.24^{b}	0.13
Starter DMI, kg/d					
Wk 7	0.83^{a}	0.72^{ab}	0.26^{ab}	0.30^{b}	0.10
Wk 8	1.80^{a}	1.61^{ab}	1.08^{b}	1.06^{b}	0.10
Rumen mass, % of BW					
Full	9.92^{a}	7.69^{b}	7.83^{b}	5.54^{c}	0.50
Empty	2.15^{a}	1.74^{b}	1.62^{b}	1.40^{b}	0.09

$^{a–c}$Means within a row with different superscripts are significantly different ($P < 0.05$).

unit. Data were analyzed as a completely randomized design with repeated measures when appropriate. Pre-planned contrasts of NOH vs. any hay, PH vs. SH, PH vs. LH, and SH vs. LH were evaluated. Calf BW, hip width and BCS were recorded at 0, 28 and 56 d and hip height at 0 and 56 d. Digestibility estimates were evaluated on d 28 using acid insoluble ash as a marker. Hip width change (5.0 vs. 4.7 cm; $P = 0.04$) and hip height change (14.0 vs. 12.3 cm; $P < 0.01$) were greater for calves fed any hay compared with calves fed NOH. No differences were observed in ADG when comparing NOH vs. any hay (1.12 vs. 1.14 kg/d; $P = 0.69$). Calves fed PH had greater ADG than calves fed LH (1.22 vs. 1.08 kg/d; $P = 0.03$). Intake (DM as %BW) was 3.0, 3.2, 3.0, and 2.8 for NOH, PH, SH, and LH, respectively, with PH greater than SH and LH ($P < 0.05$) suggesting bulkiness of the hay can limit intake and increase gut fill in young calves. Digestibility of acid (43.6 vs. 34.2%; $P = 0.01$) and neutral detergent fiber (51.3 vs. 43.3%; $P < 0.01$) were greater for calves fed any hay vs. NOH, respectively. Small amounts of processed hay should be included in the diet of weaned calves 2 - 4 mo of age, but particle length may limit DM intake.

Key Words: calf, weaned, hay particle size

M60 Effects of milk replacer plane of nutrition and levels of starch and neutral detergent fiber in pelleted starter on calf growth performance and visceral tissue measurements. T. T. Yohe*[1], T. S. Dennis[2], J. D. Quigley[2], T. M. Hill[2], F. X. Suarez-Mena[2], K. M. Aragona[2], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, [2]*Provimi, Cargill Animal Nutrition*, *Brookville, OH.*

The objectives of this study were to investigate how plane of milk replacer (MR) and feeding differing levels of starch and NDF in the starter alters visceral tissue and overall growth of the calf. The 4 dietary treatments (n = 12 per treatment) were: 691 g MR/day (dry matter basis; DM) with starter containing low or high starch (LL:12.0% and LH:35.6% starch, respectively) and 1,382 g MR/day (DM) with starter containing low or high starch (HL and HH, respectively). All calves were in individual pens with straw bedding until wk 5 when bedding was covered. Calves were fed MR twice daily (0700 and 1700 h) containing 24.5% CP (DM), 19.8% fat (DM), and had ad libitum access to pelleted starter and water. Calves arrived between 1 and 3 d of age and the study lasted 8 wk with calves undergoing step-down weaning during wk 7. Intakes were measured daily, BW were measured weekly, and calves were dissected in wk 8 for visceral tissue measurements. Data were analyzed using PROC MIXED in SAS 9.4 with the fixed effects of treatment, wk, and their interaction, and random effect of calf nested within treatment. There were treatment differences between average daily gain and starter DMI during wk 7 and 8 (Table 1). There were also treatment differences in full and empty rumen mass as percent BW at dissection (Table 1), but all other gastrointestinal measurements did not differ between treatments. These results indicate altering MR amount and starch content in pelleted starter affects ADG, starter intake and subsequently rumen growth and capacity in dairy calves around weaning.

Key Words: dairy, calf nutrition, gut development

M61 Effects of transition milk on postnatal growth and health of neonatal calves. B. Van Soest*, M. Weber Nielsen, J. Laguna, Z. Zhou, A. Abuelo Sebio, and M. VandeHaar, *Michigan State University*, *East Lansing, MI.*

Transition milk (milk from the 2nd through 4th milkings after calving) supplies additional nutrients and immunoglobulins to the calf compared with milk replacer. Our objective was to determine if feeding TM would stimulate body growth and health. Newborn calves (n = 23) received 2.8 L of colostrum before random assignment to 1 of 2 treatments (Milk Replacer, MR or Transition Milk, TM) fed 3 times per day. TM was pooled by milking number (30.3% fat (DM), 39.2% protein (DM), and 20.22 g IgG/L) and fed 1.89 L (255 g DM) per feeding as followed: milking 2 at feedings 2 to 5, milking 3 at feedings 6 to 8, and milking 4 at feedings 9 to 12 while MR calves received 275 g DM milk replacer (20.6% fat and 26.6% protein) at all 12 feedings. Health measures were scored 0 to 3 at each feeding. Body weights, growth measures, and blood samples were taken d 1, 2, 3, and 5. Both treatments had refusals of 10%. All calves had successful passive transfer of immunity with serum IgG values over 10.0 mg/mL. With the additional 0.7 Mcal ME/d, TM grew more than twice as fast at 0.64 kg/d compared with MR 0.25 kg/d ($P = 0.005$). TM tended to increase growth in heart girth and hip height (1.12 cm vs. 2.77 cm $P = 0.07$, and 0.15 cm vs. 1.45 cm $P = 0.09$). Withers height was not affected. Health scores were reduced for cough ($P = 0.05$), fecal ($P = 0.006$), nose ($P = 0.05$), and ear ($P = 0.02$) in TM calves compared with MR, with no difference observed in eye score. Blood neutrophil oxidative burst and phagocytic capacity was elevated in MR compared with TM (35.62% of cells vs 12.91% of cells $P = 0.04$; 57.64% of cells vs 48.39%, $P = 0.05$ of cells respectively). In the ileum, TM increased the number of T cells/mm of the epithelium, while no differences were seen in T cells/mm^2 of lamina propria or B cells/mm^2 of the Peyer's patches. Treatment did not affect serum IgG or total serum protein throughout the 5-d period. Feeding TM for the first 12 feedings after colostrum improved both overall growth and health in the first 5 d of life.

Key Words: calf, transition milk, development

M62 Corn processing increases starch digestibility but does not improve performance or other digestibility estimates in weaned dairy calves. T. S. Dennis*, F. X. Suarez-Mena, K. M. Aragona, T. M. Hill, and J. D. Quigley, *Nurture*

Research Center, Provimi, Cargill Animal Nutrition, *Brookville, OH*.

Previous studies in our group have observed small reductions in starch digestibility (2 to 5% units) about 1 to 4 wk after weaning when feeding whole corn (WC) vs. steam-flaked corn or a high starch complete pellet (Quigley et al., 2019; van Niekerk et al., 2020). However, we did not see overall improvements in growth as a result of increased starch digestibility which may be due to reductions in intake often seen with greater starch availability. Therefore, our objective was to evaluate performance and diet digestibility of calves fed WC from 8 to 16 wk of age compared with calves switched to processed corn at 12 wk of age. Holstein steers (n = 48) initially 58 to 60 d of age and 77.4 ± 7.6 kg BW were housed in groups (4 steers/pen) with 2 treatments randomly assigned to pens (6 pens/treatment). Treatments were a textured grower feed with WC fed for 8 wk (WCT) or WCT fed for 4 wk then switched to cracked corn for the remaining 4 wk (CCT). Grower formulas were 42% corn, 30% protein supplement, 25% whole oats, and 3% molasses (as-fed basis) with 20% CP and 43% starch (DM basis). Feeds were blended with chopped grass hay in a 95:5 ratio (as-fed basis) and offered ab libitum. Calf BW, hip widths, and BCS were recorded at 8, 12, and 16 wk of age and hip height was recorded at 8 and 16 wk of age. Digestibility estimates were evaluated at 9, 13, and 15 wk of age (before, 1 wk after, and 3 wk after diet change, respectively) using acid insoluble ash as a marker. Data were analyzed as a completely randomized design with repeated measures. Pen was the experimental unit. No differences were observed for ADG, DM intake, feed efficiency, or frame growth overall or by 4 wk period. Digestibility estimates for fat were lower at wk 13 (72.8 vs. 59.6%; $P = 0.024$) and estimates for starch tended to be greater at wk 15 (89.7 vs. 92.8%; $P = 0.052$) for CCT. No other digestibility estimates differed. In summary, increased corn processing did not improve performance in weaned calves up to 16 wk of age despite improvements in starch digestibility 3 wk after a diet change.

Key Words: weaned calf, corn processing, digestibility

M63 Impact of phase feeding Holstein calves in winter on growth and feed efficiency.

T. S. Dennis*, F. X. Suarez-Mena, K. M. Aragona, T. M. Hill, and J. D. Quigley, *Nurture Research Center, Provimi, Cargill Animal Nutrition*, *Brookville, OH*.

Calves require additional energy during the first 3 wk of life when ambient temperatures are low. Increasing total solids offered or increasing energy density in liquid feed can increase energy intake. The objective of this study was to evaluate feeding more milk replacer (MR) compared with feeding higher fat MR to achieve similar energy intakes in the first 3 wk of life during winter. Male Holstein calves (n = 98; 42.4 ± 4.7 kg initial BW; 2 to 3 d of age) were received in 2 blocks. Treatments were assigned to calves by BW at arrival and serum total protein. Treatments were: 681 g/d of 24% CP, 17% fat (as-fed basis) MR for 42 d (CON); 816 g/d of 24% CP, 17% fat MR for 21 d, then reduced to 681 g/d until 42 d (HIMR); or 681 g/d of 24% CP, 34% fat for 21 d, then switched to 681 g/d of 24% CP, 17% MR until 42 d (HIFAT). All calves were weaned by reducing MR by half from d 43 to 49. Calves were individually-housed in a barn with natural ventilation, straw bedding, and no added heat. Calf BW, hip width, hip height, and BCS were measured weekly. Temperatures were below 10°C for > 14 of the 21 d treatment period in both blocks. Data were analyzed as a completely randomized design with repeated measures, block as a random effect, and calf as experimental unit. Overall ADG, starter intake, and frame growth were similar among treatments. Calves fed HIMR and HIFAT had greater feed efficiency overall compared with CON. A treatment × time effect for ADG ($P = 0.02$), starter intake ($P < 0.01$), feed efficiency ($P = 0.05$), and medical treatments ($P = 0.09$) was observed. Calves fed HIMR gained more BW and were more efficient compared with CON and HIFAT in wk 1, and HIMR and HIFAT gained more BW and were more efficient than CON in wk 2. Starter intake was greater for CON vs. HIMR and HIFAT at wk 6 and 7. Calves fed HIMR and HIFAT had a greater number of medical treatments in wk 1 (6.1 and 5.4 vs. 1.4) compared with CON and HIMR had a greater number of treatments in wk 2 (3.4 vs. 0.9) compared with CON. Feeding more MR vs. higher fat MR appears to be a more efficient method to increase growth rates early in winter, though overall performance was similar at 8 wk of age.

Key Words: milk replacer, fat, feeding rate

M64 Carcass characteristic of dairy bull calves fed different energy sources.

E. R. Q. Vieira*[1], D. O. Cutrim[2], F. G. F. Castro[3], J. N. M. Neiva[1], H. P. S. L. Rocha[4], and R. A. Oliveira[1], [1]*Universidade Federal do Tocantins*, *Araguaína, Tocantins, Brazil*, [2]*Instituto Federal do Tocantins*, *Pedro Afonso, Tocantins, Brazil*, [3]*Agrocria Animal Nutrition and Seeds*, *Goiânia, Goiás, Brazil*, [4]*Universidade Federal de Goiás*, *Goiânia, Goiás, Brazil*.

The objective of this study was to evaluate different energy sources on quantitative carcass characteristics of confined dairy bull calves. Twenty-five, 2 mo old, Holstein x Zebu crossbred bull calves (mean BW 55.12 ± 2.2 kg) were slaughtered at 10 mo of age. Four experimental diets were used, a corn diet(whole grain corn plus pellet core), babassu mesocarp meal (BMM) diet (corn diet with 100.0 g/kg of BMM) millet diet (corn diet including 316.8 g/kg millet) and sorghum diet (corn diet including 316.8 g/kg whole grain sorghum). The animals were housed in individual pens and were randomly assigned to 1 of 4 treatments. The BMM diet provided lower ($P < 0.05$) consumption of DM, CP, NFC than the other diets, which did not differ from each other. The animals fed with the corn and sorghum diets had greater ($P < 0.05$) empty BW, and hot and cold carcass weights in relation to those fed the BMM diet, while the calves fed the millet diet did not differ in relation to the others. The animals fed with the millet diet showed lower ($P < 0.05$) hot carcass yield (50.2 kg/100 kg BW) and cold carcass yield (48.5kg/100 kg BW) compared with those submitted to the others diets. The content of the gastrointestinal tract was higher ($P < 0.001$) for those who received the diet containing millet and sorghum in relation to the diet containing BMM, while those fed the corn diet did not differ from the others. The inclusion of millet or sorghum in the form of whole grain as a source of starch in the diet for ruminants, provides carcass characteristics of the animals similar to the whole grain corn diet. The inclusion of BMM in an exclusive diet of whole grain corn reduces the development of animals and produces lighter and smaller carcasses.

Key Words: alternative feed, concentrate, ruminant

M65 Health status blood parameters in northern Italian Holstein growing calves.

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The wellbeing of growing Holstein female calves is a crucial point in dairy farms. Set up a normal blood parameters list could be important to check young herd health. The objective of this study was to investigate in growing female Holstein calves (fHc, 7–77d of life) major blood indicators of health status and their variation during this period. Nine health fHc (7 ± 2 d of life) kept at the same conditions (individual box, winter-spring period, north Italy, Grana Padano cheese production area), were enrolled and sampled at d 7, 42, 63 (weaning time) and 77. Performance parameters and blood indicators were analyzed. Dry TMR (15CP, 2EE, 28NDF, 27starch, %) and pasteurized milk (3.6fat, 3.2prot, 5lact, %; 6L/h/d) were used for feeding. A mixed model was performed to evaluate all the parameters at the time point sampled with each fHc as the experimental unit. Results showed as ADG was 600g and ave. daily TMR intake was 700g af overall the study. Table 1 shows the most relevant parameters as LSM 95% range of variability during the period sampled (7–77 d of life). In our study, we provided a list of parameters, a range of normality to help veterinarians and farmers to interpret common analysis and suggested values of health status for fHc fed daily dry TMR and 6 kg/d of pasteurized milk.

Table 1 (Abstr. M65). Most common parameters evaluated in young calves (7–77 d of life range of normality)

Parameter	LSM 95% range
Hematocrit (%)	27–38
Erythrocytes (cells/µm^3)	8.2 × 10^3–11.3 × 10^3
Leukocytes (cells/µm^3)	6.9–12.2
Platelets (cells/µm^3)	0.4 × 10^3–0.9 × 10^3
ALP (U/L)	160–440
BOHB (mmol/L)	0–0.4
Glucose (mg/dL)	70–130
Total proteins (g/dL)	5.5–7.0
Albumins (g/dL)	2.7–3.7
Globulins (g/dL)	2.4–4.5
Ca (mg/dL)	10.3–11.6
Na (mEq/L)	136–142
K (mEq/L)	4.3–5.9
Cl (mEq/L)	94–101

Key Words: Holstein calves, blood parameters, health markers

M66 Evaluation of Lifeline Protect at arrival and bovine or porcine NutraPro formulated into a traditional milk program under California feeding and housing conditions during the summer season on calf growth, morbidity, and mortality. M. Fringer*[1], J. M. Campbell[2], S. Williamson[1], and K. S. Thompson[1], [1]*California State University Fresno*, *Fresno, CA*, [2]*APC Inc*, *Ankeny, IA*.

Past studies have evaluated spray-dried bovine plasma in calves demonstrating improved growth and survival as well as reduced fecal scores and medical treatments. Minimal research has been performed using Lifeline Protect (colostrum supplement) or bovine/porcine NutraPro (spray-dried plasma) in a California rearing program during the summer in CA. The objective was to evaluate Lifeline Protect (LP) at calf arrival and NutraPro added to the milk program during California summer heat stress and document calf growth, morbidity and mortality. Calves were fed a mixture of whole milk and milk replacer fortified to 14% solids in a step-down milk program so that by day-56 calves were completely weaned off the bottle. Holstein bull calves (n = 90) were sourced from 12 different central valley dairies and transported to Fresno State University campus on June 2019. Calves were randomized to 1 of 3 feeding treatment groups based on their serum total protein level, BW and dairy origin and they were housed in wooden, California style calf hutches. The 3 feeding groups (n = 30/group) were control, porcine NutraPro (NP), and bovine NutraPro (NB). The control group d 1 first feeding was whole milk and milk replacer mix at 14% solids. For both NutraPro groups, LP was used for the first feeding then whole milk mixed with added milk replacer and either 5% NP or 5% NB to 14% finals solids. Electrolytes were given based on an as needed basis to calves that showed dehydration. Daily health and fecal scores were recorded during the AM and PM feeding. No significant differences were noted between porcine or bovine plasma in performance, morbidity, and mortality of calves. No differences were observed in calf survival between all treatments. On d 56, calf BW (75.9, 75.3, and 74.7 kg) was numerically heavier for NB and NP compared with control, respectively. Attitude and fecal scores throughout the 56 d period were reduced ($P < 0.05$) in calves fed NB or NP compared with control calves.

Key Words: calves, spray-dried plasma, calf health

M67 Effects of feeding whole milk, waste milk and pasteurized waste milk on dairy bull calf performance. S. F. Vieira[1], H. C. Diniz Neto[1], J. P. Campolina*[1], S. G. Coelho[1], and M. M. Campos[2], [1]*Department of Animal Science, School of Veterinary Medicine, Federal University of Minas Gerais*, *Belo Horizonte, MG, Brazil*, [2]*Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA), National Center for Research on Dairy Cattle*, *Juiz de Fora, MG, Brazil*.

The aim of this study was to evaluate the impact of using whole milk (WH-M), waste milk (WA-M) and pasteurized waste milk (PW-M) on the performance of dairy bull calves. Forty-five crossbred male calves (Holstein-Gir) were used with mean birth weight of 37.67 ± 5.85 kg. Immediately after birth, calves were separated from their mothers, had their navel immersed on iodine (10%), and received 10% of their BW in colostrum (minimum 25 BRIX). In the first 3 d of life, they received transitional milk. On the fourth day, they were distributed in 3 treatments: LI control (n = 15); LD (n = 15) and LDP (n = 15). On the fourth day, they were distributed in 3 treatments: WH-M (n = 15); WA-M (n = 15) and PW-M (n = 15). Six liters of milk per treatment were offered, twice a day, as well as water and starter were provided at will until the age of 57 d. The waste milk came from cows treated with antimicrobials (clinical mastitis, retained placenta and foot disease). Fast pasteurization 72 to 74 C was used for 16 s. Weight and body measurements were performed before morning meals on d 3 after birth, and weekly. The R software was used to perform the statistical analyzes, with $P < 0.05$ being considered significant. There were no differences between the WH-M, WA-M and PW-M treatments ($P > 0.05$) for final weight (76.03; 77.43 and 74.0); average daily weight gain (0.670, 0.710 and 0.620 kg / d) and body development - withers height (84.41; 84.15 and 84.28 cm), hip height (87.19; 87.35 and 87.36 cm), hip width (24.95; 25.01 and 24.82 cm) and chest circumference (86.94; 86.16 and 86.23 cm), respectively. Differences were only observed for the evaluation week ($P < 0.001$), due to the growth of the animals during this period. The use of WA-D and PW-M did not affect animals' weight gain and body development. However, further research is needed to assess the effects of the use of waste milk on the health and performance of calves in the medium and long-term.

Key Words: antimicrobial residue, body measurements, weighing

M68 Effects of feeding whole milk, waste milk, and pasteurized waste milk on ruminal parameters and gut weight on dairy bull calves. H. C. Diniz Neto[1], S. F. Vieira[1], J. P. Campolina*[1], S. G. Coelho[1], and M. M. Campos[2], [1]*Department of Animal Science, School of Veterinary Medicine, Federal University of Minas Gerais*, *Belo Horizonte, MG, Brazil*, [2]*Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA), National Center for Research on Dairy Cattle*, *Juiz de Fora, MG, Brazil*.

The aim of this research was to evaluate the impact of feeding whole milk (WH-M), waste milk (WA-M) and pasteurized waste milk (PW-M) on the concentration of volatile fatty acids (VFA) and gut weight. Forty-five crossbred bull calves (Hostein-Gir) were used with mean birth weight of 37.67 ± 5.85 kg. Immediately after birth, calves were separated from their mothers, had their navel immersed on iodine (10%), and received 10% of their BW in colostrum (min 25 BRIX). In the first 3 d of life, they received transitional milk. On the fourth day, they were distributed in 3 treatments: WH-M (n = 15); WA-M (n = 15) and PW-M (n = 15). Six liters of milk per treatment were offered, twice a day, as well as water and starter were provided at will until the age of 57 d. The waste milk came from cows treated with antimicrobials (clinical mastitis, retained placenta and foot disease). Fast pasteurization 72 to 74 C was used for 16 s. Ruminal fluid was collected at 14, 28, 42 and 56 d of age, for the evaluation of VFA: acetate (ACE), butyrate (BUT) and propionate (PRO). At 60 d of age, all animals were euthanized and had the rumen-reticulum (RR), omasum (OMA), abomasum (ABO), small intestine (SI) and large (LI) separated and weighed empty. Organs were evaluated in proportion to empty BW (EBW). The R software was used to perform the statistical analyzes, with $P < 0.05$ being considered significant. The WH-M treatment showed lower concentrations of ACE and PRO (30.49 and 20.84 mmol/L) compared with PW-M (39.75 and 28.12 mmol / L), but similar to the WA-M treatment (33.08 and 22.2 mmol/L), respectively. There were no differences between the WH-M, WA-M and PW-M treatments for BUT concentration (4.98, 5.51 and 6.25 mmol/L) and ACE:PRO (1.78, 1.76 and 1.59 µmol/mL), respectively. WH-M treatment had lower rumen weight (1.06% PV), compared with WA-M (1.26% PV) and similar weight to PW-M (1.24% PV). There were no differences between treatments in the weight of OMA, ABO, SI and LI. The use of milk with antimicrobial residue affects the ACE and PRO concentrations of and the rumen weight.

Key Words: volatile fatty acid, euthanasia

M69 The effects of two feedings of colostrum on the pre-weaning average daily gain of Holstein heifer calves. Q. Zheng*, M. Hayes, and F. Leal-Yepes, *Cornell University College of Veterinary Medicine*, *Ithaca, NY*.

Colostrum contains high concentrations of non-nutritive bioactive factors that may contribute to the healthy development of calves' gastrointestinal tract after reduced permeability to immunoglobulins, leading to their better growth. The main objective of our study was to evaluate the effect of providing an additional feeding of high quality of colostrum, corresponding to IgG ≥50mg/mL; a Brix value of ≥22.0%, 12–16 h after the first feeding on the pre-weaning average daily gain (ADG) of Holstein dairy heifers. The study was conducted from August 2019 to February 2020. Singleton Holstein heifer calves (n = 164) born unassisted with a median birth weight of 38.3kg (range: 31.7–52.6kg) that had received an initial feeding of 4L of colostrum with a median Brix of 25.4% (range: 22.0– 5.8%) within 2 h of life were selected. Calves were then randomly enrolled in blocks to either the control or treatment. The control received 3L of acidified pasteurized whole milk (Brix range: 6.1–13%), and the treatment received 3L of colostrum with a median Brix median of 25.4% (range: 22.0–33.9%). All calves were group-housed, 20 calves in each pen, with free access to pasteurized acidified milk. All calves were weighed weekly until weaning around d 63. Mixed linear models were used to asses the differences in ADG, BW, and height between the treatment and control. The mean ± SE of serum Brix % during the first week of life was 9.5 ± 0.08 and 9.1 ± 0.08 for treatment and control, respectively ($P = 0.0004$). The growth parameters measured are summarized in Table 1. A chi-squared test was generated for mortality differences among the groups. The mortality for the treatment and control group was 3.05% and 4.27%, respectively ($P = 0.54$). With the current calf management and duration of the study, the results suggested that providing an extra feeding of colostrum had no effects on the growth of Holstein heifers, however, total serum Brix is higher in the treatment group.

Table 1 (Abstr. M69).

Measurement	Control	SE	Treatment	SE	*P*-value
ADG, kg	0.68	0.02	0.68	0.02	0.94
Weaning BW, kg	77.15	1.12	77.05	1.11	0.62
Weaning height, cm	86.45	0.45	86.34	0.46	0.39

Key Words: colostrum, dairy calf management

M70 Telomere length variation and association with age and health status. T. Muratori*[1], I. Haagen[1], A. Shabtay[2], M. Cohen-Zinder[2], U. Lipkin[3], and C. Dechow[1], [1]*Penn State University*, *University Park, PA*, [2]*Newe Ya'ar Research Center, Agricultural Research Organization*, *Ramat Yishay, Israel*, [3]*The Hebrew University of Jerusalem*, *Jerusalem, Israel*.

Telomere length (TL) is a potential health and welfare biomarker that has not been fully developed in dairy cattle. The study's aim was to examine TL variation and associations with age and the occurrence of health events. TL was available for 521 observations on 434 Holsteins that ranged from birth to 97 mo of age in 2 herds. Whole blood was collected over 2 years and stored on blood cards until DNA extraction. TL was ascertained using multi-plex quantitative PCR with primers for telomere and the reference gene (β-globin) included in the same well on 384-well plates; samples were run in quadruplicate and those with a coefficient of variation greater than 5% after removing results from one outlier well were edited from the data. The difference in the number of cycles (DCt) between detection of the TL signal and reference gene signal was analyzed with a mixed model in SAS with linear and quadratic effects of age, herd, and health status as fixed effects; health status was characterized as healthy for 467 observations not associated with a health event at the time of blood collection, diseased for 32 observations where blood was collected the week of a disease treatment, and disease follow-up for 22 observations where blood was recollected 2 weeks after the initial disease collection; diseases were analyzed as one group rather than individual diseases due to the limited number of health events. Random effects were animal and collection date within herd. The repeatability of TL was 51% when calculated as the proportion of variance associated with animal relative to total variance; the standard deviation of DCt for the random animal effect was 0.82. There was a quadratic age effect with TL increasing from birth to 3 years of age and declining thereafter. The effect of health status was significant with a DCt least-squares-means of 4.70 for the diseased group which was significantly less than both healthy (5.18) and disease follow-up (5.26) groups. In conclusion, TL is a dynamic measure that appears to vary among animals, with age, and with health status.

Key Words: telomere, health, variation

M71 Comparison of fecal bacterial populations in diarrheic and healthy Holstein dairy calves from multiple farms in southeastern Pennsylvania. M. Hennessy[1], N. Indugu[1], B. Vecchiarelli[1], L. Redding[1], C. Pappalardo[1], M. Leibstein*[2], J. Toth[1], S. Garapati[3], and D. Pitta[1], [1]*University of Pennsylvania School of Veterinary Medicine*, *Kennett Square, PA*, [2]*Oceanside High School*, *Oceanside, NY*, [3]*Drexel University*, *Philadelphia, PA*.

A better understanding of the difference in the microbiome between diarrheic and healthy calves has the potential to lead to better treatment and prevention strategies for calf diarrhea. In this study, fecal samples were obtained from 10 pairs of Holstein dairy calves on 7 different farms. At each farm, 1–3 diarrheic calves (DC group) were selected (ages: 2–17 d old at sampling time) and then an age-matched (within 5 d) healthy calf (HC group) was selected as a control. Fecal samples were extracted for genomic DNA, PCR-amplified for the 16S rDNA bacteria gene, sequenced on the Illumina MiSeq platform, and analyzed using QIIME2. Number of observed species and distribution of species were similar between groups, with the DC group showing a slightly lower number of observed species and a higher Shannon diversity index than the HC group. There was a significant difference ($P < 0.05$) between farms but not between groups on unweighted UniFrac analysis; no difference was found on weighted UniFrac analysis between groups. *Firmicutes* and *Bacteroidetes* were the most prevalent phyla in all samples, with similar proportions in both groups; *Fusobacteria* were more prevalent in the DC group whereas *Proteobacteria* and *Actinobacteria* were more prevalent in the HC group. At the genus level, *Prevotella* and *Faecalibacterium* were found to be significantly ($P < 0.05$) different between the sick and healthy calves, with *Prevotella* being more prevalent in the DC group and *Faecalibacterium* being more prevalent in the HC group. *Bacteroides* was the most prevalent genus in both groups. The next most prevalent genera in the DC group were *Clostridium*, *Dorea*, *Fusobacterium*, and *Ruminococcaceae* while the next most prevalent genera in the HC group were *Lactobacillus*, *Ruminococcaceae*, *Faecalibacterium*, and *Clostridium*. Although differences were noted between DC and HC groups, these differences were not significant due to the differences between farms. Further studies are needed to identify differences between sick and healthy calves on individual farms.

Key Words: calf diarrhea, microbiome, bacterial community

M72 Pre- and post-weaning performance of calves fed milk replacer containing different sources of butyrate.

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Butyric acid, a naturally occurring fatty acid in milk, is often lacking in milk replacer formulations. We evaluated the use of a proprietary encapsulated tributyrin (TB) product against sodium butyrate (NaB) in a milk replacer formulation. Pre-weaned dairy calves (n = 41) were assigned by BW and sex to 1 of 3 treatment groups: control (C, n = 14); tributyrin (TB, n = 14); or sodium butyrate (NaB, n = 13). Milk replacers were formulated to contain 26% protein, 18% fat, and equivalent amounts of butyric acid from NaB or TB (0.8%). Calves were housed in groups, fed via an automatic feeder, with a daily allowance of 9L/day (1.35 kg of dry matter) until weaning commenced at 53 d, and milk was removed at 63 d. Water and a pelleted starter feed were offered ad libitum. Calves were weighed weekly, health scored daily for the first 3 weeks, individual milk intakes and group solid feed refusals recorded daily. Data were analyzed with ANOVA. There were no differences in calf growth rates before wean, however calves fed NaB milk replacer grew significantly less (1.45 kg/day, $P = 0.0225$, vs. 1.70 kg/day for C and 1.83 kg/day for TB groups) 2 weeks post weaning. Observation of scours was numerically greater for NaB calves during the first week, and during the trial, this group received more treatments ($P = 0.0593$). Total milk powder intake did not vary between groups ($P = 0.3846$). Pellet intake varied between groups in that during wk 4 and 5, TB group tended to consume more pellet (wk 4: 0.74 kg C, 0.70 kg NaB, 1.05 kg TB, $P = 0.0541$; wk 5: 1.103 kg C, 1.19 kg NaB. 1.50 kg TB, $P = 0.0941$), and had significantly greater intake during wk 7 (2.79 kg C, 2.31 kg NaB, 3.53 kg TB, $P = 0.0333$). While on full milk allowance (d 1 – 53), calves fed the TB milk replacer had a numerical improvement on total pellet intake (9.62kg C, 8.42kg NaB, 12.04kg TB, $P = 0.1235$). The use of a proprietary encapsulated tributyrate in calf milk replacers may improve early solid feed intake, consequently improving performance parameters at weaning, when raising calves in a group.

Key Words: dairy calf, milk replacer, butyrate

M73 Water delivery methods for neonatal calves during winter.

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Consumption of water by young calves is essential to health and growth, and is positively correlated with starter intake. However, water delivery in winter may be difficult due to freezing in some regions of the United States. Effects of water delivery method on calf intake and performance during winter was evaluated using Holstein male calves. Calves (n = 48; initially 44 ± 4.2 kg BW; 2 to 3 d of age) were randomly assigned to 2 water delivery treatments. Water treatments were either ad libitum allowance of ambient temperature water from a bucket (BUC) or 2.84 L of hot water (~38°C) offered once daily (~1200 h) for 1 h from a bottle (BOT). Water refusals from BUC were measured once daily when starter refusals were measured. Water from BOT was weighed back after the 1 h allowance period. Water treatments were applied for the first 5 wk; then all calves received ambient temperature water ad libitum from a bucket. Calves received 681 g/d of milk replacer powder (24% CP, 18% fat DM basis) split in 2 feedings for 38 d then 340 g/d for 4 d a.m. only. Milk replacer was reconstituted to 12% solids and fed in 2.84 L bottles. Textured starter was offered ad libitum (21% CP, 41% starch DM). Calves were individually housed and bedded with straw. Calves were weighed initially and weekly thereafter. Data were analyzed as a completely randomized design with repeated measures when appropriate, with calf as the experimental unit. Average temperature during the study was 3.2°C (range from −15 to 31°C). Water intake was greater ($P < 0.01$) for BOT vs. BUC (1.4 vs. 0.2 ± 0.15 SEM kg/d). Pre-weaning starter intake was similar for BOT vs. BUC (467 vs. 424 ± 77 SEM g/d), but greater ($P < 0.04$) for BOT vs. BUC post-weaning (2244 vs. 2054 ± 91 SEM g/d). Average daily gain was not affected by water delivery method. Bottle feeding warm water during the first 5 wk during winter increased water consumption which could reduce dehydration and simplify management. Feeding water from a bottle had a carryover effect on starter consumption post-weaning, which should be further investigated.

Key Words: water, bottle, bucket

M74 Effects of milk replacer feeding rate and fat content on Jersey calves to 4 months of age.

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This study evaluated effects of milk replacer (MR) feeding rate and fat concentration in MR on total-tract digestion (TTD) and performance in Jersey calves. Jersey heifer calves (n = 100 [2 blocks of 50]; initially 30 ± 3.0 kg BW; 4 to 11 d of age) were randomly assigned to 4 treatments in a 2 × 2 factorial arrangement of MR feeding rate (454 g for 42 d, then 227 g for 7 d [Low]; or 454 g for 7 d, 681 g for 35 d, and 341 for 7 d [High]) and 18 or 25% fat in MR (DM basis). Milk replacers (25% CP) were reconstituted to 14% solids and fed in 2 equal feedings for 42 d and a.m. only for 7 d. Textured calf starter (CS; 21% CP, 40% starch DM) and water were offered for ad libitum consumption. From d 57–112, CS was mixed with 5% chopped grass hay. Up to d 56, calves were housed individually and from d 57–112 in groups (4–5 calves/pen). Calves were weighed weekly from 0 to 56 d and on d 84 and 112. Frame measurements were done every 2 wk from 0 to 56 d and on d 84 and 112. Estimates of TTD were measured in 5 calves/treatment (Block 1) at wk 3 using acid insoluble ash as a marker. Data were analyzed as a completely randomized design with repeated measures, block as a random effect, and calf as experimental unit up to 56 d and pen thereafter. From 0 to 56 d, CS intake was greater ($P < 0.05$) for Low vs. High (0.76 vs. 0.64 kg/d) and 18 vs. 25% fat (0.76 vs. 0.64 kg/d); ADG was greater for High vs Low (0.56 vs. 0.52 kg/d); and hip width change was greater for 18 vs. 25% fat (4.8 vs. 4.4 cm). Estimates of OM TTD were greater ($P < 0.05$) for High vs. Low (93 vs. 87%) but NDF TTD was greater for Low vs. High (37 vs. 30%) and for 18 vs. 25% fat (39 vs. 29%). From 57 to 112 d, hip height change was greater for Low vs. High (10.0 vs. 8.8 cm) and hip width change was greater for 18 vs. 25% fat (4.6 vs. 4.2 cm). In this study, feeding Jersey calves more MR improved pre-weaning ADG but had a negative impact on starter intake and likely rumen development as fiber TTD was reduced at 3 wk and frame growth was reduced from 8 to 16 wk. No benefits were observed for feeding more fat in MR as starter intake, fiber digestibility, and frame growth were reduced.

Key Words: feeding rate, digestibility, Jersey calf

Lactation Biology 1

M75 Sodium salicylate reduced transcript abundance of hypoxia-associated genes in MAC-T cells. C. M. Ylioja, T. H. Swartz, L. K. Mamedova*, and B. J. Bradford, *Kansas State University*, *Manhattan, KS*.

Hypoxia is an oxygen deficiency commonly found in growing tissues and is speculated to occur in the rapidly developing mammary gland in peripartum dairy cattle. Low oxygen concentrations can activate hypoxia-inducible factor-1 (HIF-1), which increases transcription of genes involved in angiogenesis (*VEGF*) and glucose transport (*GLUT1*). The mRNA stability of these genes is positively regulated by AUF1. In our previous research, postpartum administration of sodium salicylate (SS) increased whole lactation milk yield in multiparous cows but tended to reduce milk yield in primiparous. Because rapid mammary tissue development likely occurs in cows approaching first lactation, we hypothesized that SS inhibited the activation of HIF-1α and decreased transcription of downstream targets. MAC-T cells were treated with SS (100 μM) or control media before incubation under either hypoxic (1% O_2) or normoxic conditions for 12 h. Additionally, cells were transfected with either HIF1α siRNA or a scrambled siRNA negative control 48 h before hypoxia treatments. *HIF1α*, *GLUT1*, *VEGF*, and *AUF1* were quantified using the $2^{-\Delta Ct}$ method and normalized to the internal control gene *NENF*. Transcript abundance was assessed using a linear mixed model with the fixed effects of SS, hypoxia, and siRNA and all 2- and 3-way interaction terms, and the random effect of plate nested within hypoxia. SS tended to decrease *HIF1α* as compared with untreated cells ($P = 0.09$). For *GLUT1*, SS treatment interacted with hypoxia ($P = 0.05$), as SS reduced *GLUT1* when MAC-T cells were cultured in normoxic conditions ($P < 0.01$), however, no effect of SS was found in hypoxia-treated cells ($P = 0.39$). Regardless of oxygen status, SS reduced *VEGF* ($P = 0.04$) and *AUF1* ($P = 0.04$) relative to untreated cells. Hypoxia increased *GLUT1* ($P = 0.01$), yet no effect was identified on *VEGF* ($P = 0.45$) or *AUF1* ($P = 0.22$). siRNA knocked down *HIF1α* ($P < 0.01$), but no effect was found on *GLUT1* ($P = 0.98$), *VEGF* ($P = 0.99$), or *AUF1* ($P = 0.62$). In conclusion, SS reduced transcript abundance of genes involved with mammary gland development, but generally did not interact with oxygen status.

Key Words: hypoxia, NSAID, mammary gland development

M76 Circadian *PER2* gene silencing suppresses lipid synthesis partly via inhibition of *PPARG* and *SREBF1* in bovine mammary epithelial cells. Y. J. Jing[1], Y. F. Chen*[1], M. Z. Wang[1], L. Y. Hu[1], Q. Y. Xu[1], Z. N. Xi[1], and J. J. Loor[2], [1]*Yangzhou University*, *Yangzhou, Jiangsu, China*, [2]*University of Illinois*, *Urbana, IL*.

In non-ruminants it is well-established that biological rhythms play a profound role in coordinating whole-body metabolism. In dairy cows there is evidence that milk yield and milk fat content have rhythmic pattern thought to be regulated by circadian rhythms. The core circadian clock gene period 2 (*PER2*) is associated with mammary gland development and lipid synthesis in rodents, partly via regulating peroxisome proliferator-activated receptor gamma (PPARG). Whether such type of molecular link between circadian clock and lipid metabolism exists in bovine is unclear. We hypothesized that PER2 is associated with lipid metabolism in bovine mammary cells. To test this hypothesis, the bovine mammary tissue samples were obtained from 3 mid-lactation (averaged 110 d postpartum) cows and digested by collagenase to gain the primary bovine mammary epithelial cells (BMECs). Small interfering RNA (siRNA) technology was used to inhibit PER2 expression in primary BMECs. The primary BMECs were transfected with 3 siRNAs at 0, 12, 24, 36, 48, 60 h to screen out the best siRNA and its transfection time point. The lipid droplet was measured by red oil O staining, and the triacylglycerol (TAG) content of BMEC was determined with the tissue triglyceride assay kit (APPLYGEN, China). The lipid droplet and TAG content were determined at 36 h (36 h showed the greatest PER2 gene inhibitory effect of 84.7%) after the siRNA transfection. One-way ANOVA and Duncan's multiple comparison were used to conduct statistical analysis by SPSS software version 22.0 (statistical significance set at $P < 0.05$). Silencing of PER2 led to lower concentration of cellular lipid droplets and TAG levels in BMECs ($P < 0.05$). In addition, PER2 silencing downregulated mRNA of *ACACA*, *FASN*, *LPIN1* and *SCD* ($P < 0.05$), indicating an overall inhibition of lipogenesis and desaturation. The downregulation of PPARG and SREBF1 in response to PER2 silencing underscore the importance of circadian clock signaling and transcriptional regulation of lipogenesis. Therefore, data suggest that PER2 participates in the coordination of mammary lipid metabolism and may be a component of the control of lipid droplet and TAG synthesis in mammary cells.

Key Words: *PER2* silencing, mammary epithelial cell, lipid metabolism

M77 Milk fatty acid profiles of beef cows in response to a short feed restriction during lactation. I. Casasús*, J. R. Bertolín, K. Orquera, J. Ferrer, and M. Blanco, *Ctr Invest y Tecnol Agroal Aragon (CITA), IA2 (CITA-Universidad de Zaragoza)*, *Zaragoza, Spain*.

The relationship between energy balance and the milk fatty acid (FA) profile is well established in dairy cows but has received little attention in beef cattle. We analyzed the milk fatty acid profile of 16 Parda de Montaña beef cows 2 mo post-calving in response to a 4-d (d) dietary restriction (55% of energy requirements, 6.2 kg dry matter (DM) hay/d), as compared with a previous basal and an 8-d refeeding period (100% of requirements; 7.0 kg DM/d hay + 2.7 kg DM/d concentrate). With d0 as the start of restriction, milk was sampled on days d-2 (basal), d1, d3 (restriction) and d5, d6, d8 (refeeding). Individual FA were identified by gas chromatography, and sums of FA were calculated (saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), cis-MUFA, trans-MUFA, C4-C15 de novo synthesis FA and C16-C24 mobilization FA). These sums and the 4 major FA (C16:0, C18:1–9c, C18:0, C14:0) were analyzed using mixed models, with day as fixed and cow as random effects. All the results presented here were significant at $P < 0.001$. The milk FA profile responded immediately to changes in the energy balance and/or the diet. On d1 of restriction, the concentrations of SFA decreased, mainly due to a reduction in the de novo synthesis FA and C16. A concomitant increase in MUFA (associated with that of C18:1–9c, predominant in body fat) was observed. These changes, along with the increments in C16-C24 FA, indicate an enhanced fat mobilization from the adipose tissue. During the restriction, C18:0 and trans-MUFA decreased while *cis*-MUFA and PUFA increased, as a result of both the mobilization and the change in diet composition. The opposite occurred in the refeeding phase. On d5, MUFA decreased (due to the reduction in C18:1–9c) and SFA increased because of the rise in the de novo synthesis FAs and C16:0, reflecting the reversion of fat mobilization. At the end of refeeding (d8), the individual FA returned to basal concentrations, but the sum of C16-C24 mobilization FAs was even lower and that of C4-C15 de novo synthesis FAs was higher than basal values, indicating a possible "rebound effect" after restriction and refeeding.

Key Words: beef cows, nutritional challenge, milk fatty acid profile

M78 Effects of glucose and acetate infusion on mammary uptakes of essential amino acids by lactating dairy cows. B. Li*[1], R. Laforest[1], L. Wright[1], J. Kim[1], P. Kedzierski[1], V. Osborne[1], J. Doelman[1,2], and J. Cant[1], [1]*University of Guelph*, *Guelph, ON, Canada*, [2]*Trouw nutrition*, *Putten, the Netherlands*.

Previous research suggests that glucogenic energy can stimulate milk protein yield of dairy cows while lipogenic energy does not. To explore differences in mammary essential amino acid (EAA) utilization between these types of energy, 5 rumen-fistulated cows were given additional glucose or acetic acid in a 5 × 5 Latin square design. Infusion treatments were:

1) 10 L/d ruminal water (CTL), 2) 1.1 kg/d (LoG) or 3) 2.2 kg/d (HiG) abomasal glucose, and 4) 1.25 kg/d (LoA) or 5) 2.5 kg/d (HiA) ruminal acetic acid. Acetic acid and glucose infusion rates were isocaloric at low and high levels, respectively. Milk yields were recorded daily and milk samples were collected on the last 3 d of each 7-d infusion period. Tail and mammary venous samples were collected on d 7 to estimate mammary uptakes. Plasma samples for each cow were pooled over time by period. Plasma AA concentrations for each time point were analyzed using Ultra Performance Liquid Chromatography in conjunction with Empower Chromatography Data Software (Waters Corporation, Milford, MA). Linear contrasts of glucose and acetic acid dose were estimated by ANOVA assuming fixed effects of period and treatment, and random effects of cow. Glucose infusion had no effect on DMI ($P = 0.97$) or milk protein yield ($P = 0.15$) but increased lactose yield ($P = 0.03$) and tended to increase milk yield ($P = 0.07$). Acetic acid infusion dramatically decreased DMI from 18.8 kg/d on CTL to 13.8 and 14.9 kg/d on LoA and HiA respectively. Milk yield decreased 5.4 kg/d, protein yield decreased 201 g/d and lactose yield decreased 224 g/d on HiA compared with CTL ($P \leq 0.01$) due to the decrease of DMI. Glucose infusion decreased arterial concentrations of all EAA ($P < 0.01$) except Met and Thr, but increased mammary plasma flow (MPF) rate ($P < 0.01$), so that mammary uptakes of EAA were not affected ($P > 0.16$). In contrast, acetic acid infusion increased concentrations of Ile, Leu, and Val ($P < 0.08$) without affecting other EAA concentrations ($P > 0.20$), had no effect on MPF ($P = 0.70$), and decreased mammary uptakes of Arg, Ile, Leu, and Phe ($P < 0.10$). Findings suggest that exogenous glucose encouraged milk protein production despite reduced plasma concentrations of some EAA, while exogenous acetic acid discouraged milk protein yield thereby increasing concentrations of some EAA.

Key Words: cow, amino acid, milk protein

M79 Effect of methionine in membrane traffic for milk secretion in the goat's mammary epithelial cell.

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Methionine (Met) supplementation increases milk, protein and fat yields in cow. We investigated whether this could be partly explained by an increasing flow of milk components in the secretory pathways of mammary epithelial cells. Multiparous Alpine goats at mid lactation (n = 48), grouped by levels of expression of the *CSN1S1*, were assigned to 4 treatments in a randomized complete block design. Goats were fed a fixed amount of hay and a low (LE, 1.47 Mcal/kg DM) or adequate (AE, 1.54 Mcal/kg DM) energy concentrate combined with 2 levels of metabolizable Met: unbalanced vs. balanced using isopropyl ester of 2-hydroxy-4-methylthio butanoic acid (HMBi 0.24% concentrate DM) to cover 100% of Met requirement, based on cow requirement (INRA, 2007). Treatments were: LE, LEMet (LE, balanced Met), AE and AEMet (AE, balanced Met) for 5 weeks. Goats (23) were slaughtered and mammary tissue was processed for Western blotting using secretory compartment specific markers of membrane traffic. Milk protein yield ($P = 0.01$) and casein content ($P = 0.01$) increased in goats fed the Met balanced diets. The amount of the endoplasmic reticulum (ER) markers, Cnx and ERLIN2, decreased (20%, $P \leq 0.05$) in goats fed the LE diet. Met balanced diets had the opposite effect on both markers (20%, $P \leq 0.05$), as well as on protein disulfide isomerase (45%, $P \leq 0.05$). These observations are in agreement with a positive effect of Met on the activity of the ER, the site where protein and lipid are synthesized. On the other hand, a specific marker of the exit site of the Golgi apparatus and secretory vesicles formation (AP1) decreased with the LE diet (25%, $P \leq 0.05$) and its highest level was found in goats fed Met balanced diet at AE supply. The higher ßCOP ($P = 0.01$), a marker of intra Golgi transport, clearly reflected a decrease in membrane transport of LE diets. These data strongly suggest that energy level has a direct impact on membrane traffic in the secretory pathway of mammary epithelial cell while Met improve ER activity and has the tendency to further promote intracellular transport of milk components and, ultimately, their secretion.

Key Words: amino acid supplementation, dairy goats

M80 Effect of heat stress during the dry period on estradiol and prolactin interactions in mammary gland gene expression of Holstein cows.

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The dry period, a 6 to 8-week nonlactating state between lactations, is essential for maximal mammary development and lactation in dairy cows. Although late-gestation heat stress decreases estrogen (E) and increases prolactin (PRL) concentrations in blood, those impacts on mammary development remain unclear. The objective of this study was to determine how late gestation heat stress-induced E and PRL alterations affect the expression of their receptors and signaling in the mammary gland at different stages of the dry period. Fourteen cows were either exposed to in vivo heat stress (HT, n = 7) or active cooling by fans and soakers (CL, n = 7) for the entire dry period (~45 d). Mammary gland biopsies were performed on d 3 (i.e., involution) and 35 (i.e., proliferation) relative to dry off and equally divided in 3 explants, that were incubated in vitro for 24h in 1 of the 3 mediums: 1- Basal (Bm no PRL or E); 2- CL mimic (Cm: basal + 20ng/mL PRL + 5.8ng/mL E; and 3- HT mimic (Hm: basal + 40 ng/mL PRL + 2.9ng/mL E). Gene expression of *PRLR-SF*, *PRLR-LF*, *ESR1* and *ESR2* were measured using Real Time qPCR. An ANOVA using the mixed procedure of SAS was performed to assess the impacts of in vivo (HT, CL), in vitro (Bm, Cm, Hm) treatments and their interaction on relative transcript expression. Dry cows subjected to HT had increased rectal temperature and respiration rate relative to cows subjected to CL (39.1 vs 38.8 ± 0.01°C and 65.2 vs 55.4 ± 1.2 breaths/min, respectively), which confirms cooling conditions are effective and necessary for the thermal equilibrium of the CL cows. *In vivo* HT increased the expression of *PRLR-LF* relative to CL. However, Hm in vitro treatment decreased the expression of *PRLR-SF, ESR1* and *ESR2* relative to Bm treatment. These results suggest that E and PRL alterations caused by heat stress exposure can modulate the expression of receptors in the mammary gland, with potential implications for normal mammary development during the dry period.

Key Words: heat stress, mammary explants, culture

M82 Evaluation of breed and udder characteristics on somatic cell count and udder pathogens in lactating Holstein and Jersey cows.

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The objective of this study was to evaluate the impact of physical udder characteristics and breed on hygiene scores (HS), milk yield (MY), conductivity (COND), SCC, and bacterial cultures (BC) in lactating Holstein and Jersey dairy cows housed in a compost-bedded pack barn. Lactating Holstein and Jersey (n = 10 each) cows were evaluated during a 6-wk period. Milk samples were collected as a sterile composite from all 4 quarters during one milking/wk and SCC was determined using the DeLaval Cell Counter DCC. If SCC $\geq$350,000 cells/mL, the sample was cultured to determine bacterial species. Milk samples were cultured using a Tri-plate agar including Factor, MacConkey, and Focus media (University of Minnesota Easy Culture). Cows were evaluated once/wk using a multi-zone hygiene scoring system for udder cleanliness (1 = very clean to 4 = very dirty; Cook, 2002). Udder measurements were taken during wk 1 and included udder depth and circumference, and teat length. Milk yield and COND were measured daily and averaged by wk using the AfiMilk parlor system (Afimilk, Kibbutz Afikim, Israel). Statistical analysis of MY, COND, and udder measurements were conducted using the MIXED procedure in SAS (v9.4). Analysis of HS incidence and BC species counts were evaluated using the FREQ procedure in SAS (v9.4). No differences in BC, SCC, or physical udder characteristics were observed among breeds. Holstein cows produced more milk than Jerseys (37.6 and 26.5 ± 3.01 kg/d, respectively; $P = 0.0181$) and had greater COND (9.70 and 8.81 ± 0.15 mS/cm, respectively; $P = 0.0005$). Jerseys exhibited improved udder (64.4 vs. 25.4% score 1 and 28.8 vs. 64.4% score 2, respectively; $P = 0.0003$) and flank (61.0 vs. 22.0% score 1; 35.6 vs. 57.6% score 2; and 3.4 vs. 18.6% score 3, respectively; $P = 0.0001$) HS more frequently than Holsteins, indicating that Jersey udders and flanks were overall cleaner than Holsteins. Jerseys may be better suited for compost-bedded pack barns than Holsteins based on the observed improvements in conductivity and hygiene scores.

Key Words: udder measurements, breed, SCC

M83 Relationships of somatic cell count with milk lactose and protein over the first 10 days postpartum in dairy cows. H. Peterson*[1], T. Kelley[1], J. Williams[1], W. Price[3], E. Peterson[4], M. McGuire[2], and M. McGuire[1], [1]*Animal and Veterinary Sciences, University of Idaho, Moscow, ID*, [2]*Margaret Ritchie School of Family and Consumer Sciences, University of Idaho, Moscow, ID*, [3]*Statistical Programs, College of Agricultural and Life Sciences, University of Idaho, Moscow, ID*, [4]*Erik L. Peterson Dairy, Filer, ID*.

There is little research examining the relationships of somatic cell count (SCC) with lactose and protein concentrations in bovine milk. Understanding these relationships may provide insight into how animal health is related to milk composition and quality. The objective of this study was to examine the relationships of SCC and lactose and protein in bovine milk in the first 10 d postpartum. Quarter-milk samples were collected daily from 107 cows on 4 dairies and milk components assessed by near-infrared analysis. Data were analyzed using a mixed linear regression model with SCC (log-transformed) and day postpartum as independent variables and dairy as a random variable while assuming a compound symmetric correlation structure for the repeated measures of day within quarter of each cow. Milk type was characterized as colostrum (d0; C), transitional milk (d1-d4; TM), and mature milk (d5-d10; MM). Lactose concentrations were 3.16 ± 0.03%, 4.08 ± 0.01%, and 4.32 ± 0.01% in C, TM, and MM, respectively; protein concentrations were 9.40 ± 0.10%, 3.94 ± 0.03%, and 3.03 ± 0.01% in C, TM, and MM, respectively. The range of SCC across all samples was 1,000 to 9,999,000 cells/mL. In C, there was no relationship between SCC and lactose, but there was a negative relationship between SCC and protein ($P = 0.0004$). For TM and MM, there was a negative relationship between SCC and lactose that became more negative as days postpartum progressed. There was a positive relationship between SCC and protein in TM; conversely, there was a negative relationship between these variables in MM. For both TM and MM, protein decreased with increasing days postpartum. These results suggest relationships of SCC with lactose and protein exist; however, they are not consistent over time.

Key Words: dairy cow, milk, somatic cell count

M84 Evaluation of mammary gland involution in dairy cows during the dry period using a 3-dimensional scanner. J. Leite de Campos*, J. Strickland, J. Gandy, L. M. Sordillo, C. Robison, and P. L. Ruegg, *Michigan State University*, *East Lansing, MI*.

Mammary gland involution is an important process during the dry period, and it is associated with immune defenses and the capability to prevent intramammary infection that could affect subsequent lactations. Currently methods to evaluate udder involution during the dry period are limited. The objective of this research was to compare the use of a 3D scanner and a metric tape to measure mammary gland involution in dairy cows. Clinically healthy Holstein cows (n = 15) of parity 2 through 5 were included in the study if they had a SCC < 200.000 cells/mL at the end of lactation. Mammary gland dimensions were measured at the dry off (d 0), and 2, 7, and 14 d later using both a 3D Scanner (Structure Sensor, Occipital, San Francisco) and manual measurements of marked areas between the middle rear left and right quarters and the median suspensory ligament. Data was collected from November 2019 – February 2020. Files obtained from the 3D scanning were analyzed for surface area of the rear quarters using 3-Matic software (Materialise, Belgium). Percentage change was calculated between measurements obtained at d 0, and measurements taken at d 2, 7, and 14. Analysis of variance was performed to analyze data. When measured manually using a metric tape, the width of the quarters increased and then decreased and were 117%, 101%, and 93% on d 2, 7, and 14, respectively. When measured using a 3D scanner, the surface area of the rear quarters was 125%, 100%, and 89% on d 2, 7 and 14 respectively. No significant difference in measurements was found based on method ($P = 0.88$). When width was measured manually, an increase in 16% and a decrease in 24% was observed from d 2 to 7 and d 2 to 14 ($P < 0.01$). When surface area was measured using the 3D scanner, it increased 25% and decreased 36% between d 2 to 7 and d 2 to 14, respectively ($P < 0.001$). No significant change in mammary gland dimensions was observed from d 7 to 14 ($P > 0.08$). Changes in mammary gland dimensions during the dry period were quantifiable using either manual measurements or 3-D scanning.

Key Words: dry period, udder involution, 3-dimensional scanner

M85 Potential of nanoparticles containing matrix metalloproteinase-9 (MMP-9) as a dry-off enhancer: Pulling apart the effects of MMP-9 and nanoparticles. L. Gifre-Renom[1], J. V. Carratalá[2], S. Parés[1], L. Sanchez-Garcia[2], N. Ferrer-Miralles[2,3], A. Villaverde[2,3], A. Bach*[4,1], E. Garcia-Fruitós[1], and A. Arís[1], [1]*Department of Ruminant Production, Institut de Recerca i Tecnologia Agroalimentàries (IRTA*, *Caldes de Montbui, Catalonia, Spain*, [2]*Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona*, *Cerdanyola del Vallès, Catalonia, Spain*, [3]*Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona*, *Cerdanyola del Vallès, Catalonia, Spain*, [4]*Institució Catalana de Recerca i Estudis Avançats (ICREA)*, *Barcelona, Catalonia, Spain*.

The dry period is a non-milking interval when the mammary gland involutes and regenerates to guarantee an optimal milk production in the subsequent lactation. Several bottlenecks such as the high risk of intramammary infections may hamper this process. Antibiotics have been routinely used as a preventive treatment, but the concerns about potential antibiotic resistance calls for alternative preventive strategies. Matrix metalloproteinase 9 (MMP-9) is an enzyme able to degrade the extracellular matrix, triggering the involution and immune function of the mammary gland. Thus, the objective of this study was to determine in vivo whether the involution and immune function enhancement previously observed after the administration of inclusion bodies (IB) containing MMP-9 was due to the nanoparticle format or to the inherent properties of the MMP-9 comprised in IB. Eight cows were enrolled in this study and 30 quarters of these cows were considered the experimental unit (4 quarters per cow in 7 cows, and 2 quarters in 1 cow). A dose of 1.2 mg of both active and inactive MMP-9 IB and 10 mL of saline solution were infused into 10 quarters per treatment at dry-off. At 1, 3, 6, and 9 d after protein infusions, mammary gland secretions (MGS) were obtained and analyzed for SCC, immune cell populations, BSA, lactoferrin, Na^+/K^+, and endogenous MMP-9. Data were analyzed using a fixed-effects model. There were only minor differences in the parameters monitored between the infusion of active or inactive rMMP-9 IB. Briefly, concentration of BSA in MGS were greater at 1 and 6 d in quarters treated with active rMMP-9 IB ($P < 0.01$) than those treated with inactive IB. Similarly, the Na^+/K^+ ratio in MGS increased at d 6 and was sustained at d 9 ($P < 0.01$) with the active MMP-9 IB compared with the inactive IB. Thus, the minor differences triggered by the administration of an active or an inactive form of MMP-9 led to conclude that the response observed in the bovine mammary gland was mainly due to the protein format (nanoparticle or soluble) but not to the biological activity of the MMP-9 embedded in the IB. This study provides relevant information on the future use of protein IB in the mammary gland of cows and the role of MMP-9 at dry-off.

Key Words: dry period, inclusion body, mammary gland

M86 Mitochondrial function in the liver and skeletal muscle of mid-lactation dairy cattle. V. R. Favorit*[1], A. N. Kavazis[2], W. R. Hood[2], P. Villamediana[1], and A. L. Skibiel[1], [1]*University of Idaho*, *Moscow, ID*, [2]*Auburn University*, *Auburn, AL.*

Most energy produced in the cell is through oxidative phosphorylation (OXPHOS) and mitochondrial activity changes according to cellular energy demand. At peak lactation, mammary OXPHOS machinery and ATP production are upregulated in association with increased mammary energy requirement and milk output. It is unclear if concurrent shifts occur in tissues with supporting roles in milk synthesis, such as skeletal muscle and liver. We assessed relationships between milk production and measures of mitochondrial function in these tissues at mid-lactation. Liver and skeletal muscle biopsies were taken from multiparous Holstein cows (n = 11) in mid-lactation (75 ± 4 d). Milk yield was recorded daily to 80 d in milk (DIM) and milk samples collected for composition analysis (fat, protein, lactose) at 74 DIM. Mitochondria were isolated and oxygen consumption measured in a respiration chamber. Respiratory control ratio (RCR) was used as a measure of the functional and coupled state of mitochondria and calculated as the ratio of maximal ADP-stimulated respiration to basal respiration following ADP phosphorylation using either complex I (NADH-linked) or II ($FADH_2$-linked) substrates. Mitochondrial emission of reactive oxygen species (ROS) was also measured. Correlation analysis was used to examine relationships between mitochondrial measures and average milk yield from 40 to 80 DIM (mid-lactation) only and from 5 to 80 DIM (i.e., early-to-mid). Liver complex II RCR at mid-lactation was positively correlated with early-to-mid milk yield, signifying increased fat substrate utilization to produce ATP and minimal proton leak ($r = 0.74$, $P = 0.01$). No association was observed between milk components and mitochondrial RCR or ROS. Mid-lactation milk yield was positively correlated with skeletal muscle mitochondrial ROS production ($r = 0.66$, $P = 0.05$) and tended to correlate with liver mitochondrial ROS production ($r = 0.63$, $P = 0.1$). Our results suggest that elevated energy demands associated with milk production are met with an increased efficiency of mitochondrial ATP production in liver but result in greater oxidant emission in skeletal muscle and liver.

Key Words: oxidative phosphorylation, lactation, metabolism

M87 An optimized laser capture microdissection protocol for intact RNA isolation from lipopolysaccharide treated mammary epithelial cells. R. K. Choudhary*[1], T. B. McFadden[2], E. M. Shangraw[2], R. O. Rodrigues[2], and F.-Q. Zhao[1], [1]*Department of Animal and Veterinary Sciences, University of Vermont*, *Burlington, VT*, [2]*Division of Animal Sciences, University of Missouri*, *Columbia, MO.*

Laser capture microdissection (LCM) is one popular technique for isolating specific cell types from tissues. However, RNA quality, quantity and integrity in LCM samples can be greatly affected by tissue treatment, length of dissection and the total areas of cells dissected. In this study, we optimized methodology to obtain high quality RNA from mammary epithelial cells collected from bovine mammary glands treated with lipopolysaccharide (LPS) and determined if LPS affected the quality, quantity and integrity of RNA. Ten multiparous cows were used. Five treatment (T) cows received one intramammary dose of LPS (50 µg in 10 mL saline; TL) in each of 2 ipsilateral glands while the contralateral glands received saline (10 mL; TS). Likewise, in 5 control (C) cows, saline (CS) was infused into 2 ipsilateral glands and the other glands remained uninfused (CU). Mammary tissues were collected at 0, 3 and 12 h, relative to infusions and processed for LCM. After staining, tissue sections were visualized using an epifluorescence microscope with attached computer and manually selected areas of epithelial cells were dissected using LCM. Time of dissection was kept minimal (13.6 + 0.52 min; mean + SE) to avoid RNA degradation, and areas of dissected cells were consistent across treatment groups and times. Results showed that fixation of tissue sections with chilled 70% ethanol, histogene staining (with RNase inhibitor), dehydration in absolute ethanol, and final clearing in xylene was able to preserve quality of RNA isolated from microdissected cells. Analysis of total RNA from mammary epithelial cells harvested by LCM showed RNA yield per unit area was affected by treatment, time and interaction of treatment x time, suggesting that LPS increased transcription or reduced RNA degradation in epithelial cells. In addition, RNA integrity number (RIN) was affected by treatment and time x treatment interaction ($P \leq 0.01$). In summary, we developed an optimized LCM protocol to reproducibly obtain high-quality RNA and suggest that LPS treatment may affect RNA yield of mammary epithelial cells.

Key Words: mastitis, RNA quality, transcription

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M88 Environmental effects on teat microbial population of transition dairy cows housed in compost bedded pack barns. T. L. France*, J. C. H. Costa, and M. C. Morgan, *University of Kentucky*, *Lexington, KY*.

Compost bedded pack barns utilize composting methods which require microbial growth. In turn, this may increase the exposure of mastitis-causing pathogens to the teats of dairy cows. Our objective was to determine the relationship between environmental effects and cow bacteria counts over time in transition cows. Twenty-six Holstein cows (11 primiparous, 15 multiparous) were enrolled in an observational study from December 2018 to May 2019. Quarter teat skin swabs, teat end swabs and milk samples were aseptically collected from every cow 14 d before expected calving date, 3 d postpartum, and biweekly through 60 ± 7 DIM. Bedding samples were collected weekly (n = 396). Teat skin swabs, teat end swabs and bedding samples underwent microbial analysis to enumerate environmental microbes. Bedding samples were analyzed for moisture, C:N ratio and pH. Milk samples were cultured for presence or absence of bacteria. Pearson correlations determined associations among cow variables. Linear mixed models determined effect of stages in the transition period (expt week) and environmental factors on cow bacteria counts. Teat skin total bacteria count (TBC) had a moderate relationship with teat end TBC ($r = 0.42$; $P < 0.001$). Teat end TBC had a weak relationship with milk TBC ($r = 0.18$; $P < 0.001$). Expt week affected all cow bacteria counts ($P < 0.05$). Parity affected milk TBC and teat skin counts ($P < 0.05$). Teat skin coliform and *Staphylococcus* spp. (Staph.) counts changed ($P < 0.05$) from pre- to postpartum, where LS Means of coliforms increased from 2.09 to 2.75 $\log_{10}$ cfu/g and Staph. counts decreased from 5.67 to 4.53 $\log_{10}$ cfu/g. Similar results were found for teat end counts, where LS means of coliforms increased from 1.19 to 1.46 $\log_{10}$ cfu/g and Staph. counts decreased from 4.08 to 2.63 $\log_{10}$ cfu/g. Bedding moisture was the only factor that significantly affected teat skin bacteria counts. As moisture increased by 1%, teat skin TBC decreased by 1.06 (0.59; SE) $\log_{10}$ cfu/g. We conclude that environmental factors over time do not influence teat microbial population. Calving time may be a larger contributing factor to teat microbiome.

Key Words: bacteria count, mastitis

M89 Distribution of seasonality of milk yield, adjusted for parity and days in milk, according to heat stress intensity in the United States. A. P. S. Franzoni*[1], F. C. Ferreira[1], J. S. Clay[2], and A. De Vries[3], [1]*University of California*, *Davis, CA*, [2]*Dairy Records Management Systems*, *Raleigh, NC*, [3]*University of Florida*, *Gainesville, FL*.

The objective of this study was to describe the distribution of seasonality in milk yield, adjusting for parity and days in milk, under different intensities of heat stress (HS) in the United States (US). We used 2015 DHIA lactation records from 5,005 herds located in 41 states of the US. Weather data of closest station of each herd was retrieved from the National Oceanic and Atmospheric Administration archives. Linear regression models with sine and cosine functions adjusted for days in milk and parity were used to describe seasonality in average milk production per herd, and to find the annual low (minimum) and peak (maximum) of the curves. Annual low-to-peak (LP-ADJ) ratios were measures of maximum seasonality. Sine and cosine functions were also used to model hourly and daily temperature humidity indexes (THI) for each weather station. For every herd, we calculated the average daily THI and the number of hours per day above a THI of 68. Calculated measures of HS intensity were the yearly heat load (HL), the number of days per year with average THI above 68 (DAT), and the number of hours per year above 68 (HAT). For each HS intensity measure, herds were classified as exposed to LOW (HL <73, DAT <50, HAT < 1108), MEDIUM (HL >73 and <299, DAT >50 and <86, HAT > 1108 and < 1778), and HIGH (HL > 300, DAT > 87, HAT >1779) intensity HS groups. The PROC CORR procedure (SAS 9.4) revealed correlation (<0.001) between LP-ADJ and HL ($r = 0.19$), DAT ($r = 0.16$), and HAT ($r = 0.17$). Distribution of seasonality (25, 50, and 75 percentiles) for LOW, MEDIUM and HIGH HS intensity were: HL (0.88; 0.92 and 0.94), (0.88; 0.92 and 0.95) and (0.85; 0.90 and 0.94); for DAT (0.89; 0.91 and 0.95), (0.89; 0.91 and 0.95) and (0.86; 0.89 and 0.94); and for HAT (0.89; 0.91 and 0.95), (0.89; 0.92 and 0.95) and (0.86; 0.90 and 0.94). Herds exposed to HIGH HS had a greater variation in their seasonality, but HS intensity was not a major effect in seasonality variation among herds. Even within herds exposed to HIGH HS, seasonality can be managed and reduced, for instance, through efficient heat abatement strategies.

Key Words: seasonality, dairy cow, sine and cosine functions

M90 Mammary health program and subclinical mastitis in dairy farms in Córdoba, Argentina. C. Vissio[1,2], B. Mancilla[1], P. Turiello*[1], and A. Larriestra[1], [1]*UNRC*, *Río Cuarto, Córdoba, Argentina*, [2]*IDAS UNRC-CONICET*, *Río Cuarto, Córdoba, Argentina*.

The aim of this study was to evaluate the knowledge and the application of the main practices on the mammary health program (MHP) and their association with the incidence of subclinical mastitis (SM) in dairy farms in Córdoba, Argentina. The study was conducted among producers who had information about somatic cell count (SCC) of at least 6 official milking tests during the years 2017 and 2018. Producers were invited to answer an online survey about practices and knowledge related to MHP. Once the information was gathered, a mixed model with repeated measurements was run, considering as main predictors those MHP practices and including herd size as an adjustment factor. The response variable was SM incidence, determined as the proportion of cows whose SCC increased from <200,000 to ≥200,000 cells/mL over 2 consecutive test days. The statistical analysis was performed on STATA. Of the 83 eligible producers, 61%

Table 1 (Abstr. M90). Results of the final model on the association between practice of MHP and herd SM incidence in dairy farms of Córdoba, Argentina

Variable	Category	Estimator (SE)	*P*-value	Incidence (95% CI)
Extra pen	Yes	−0.09 (0.03)	0.005	0.20 (0.18–0.23)
	No			0.29 (0.24–0.35)
Pre-dipping	Yes	−0.08 (0.02)	0.001	0.19 (0.17–0.22)
	No			0.27 (0.23–0.31)
Foremilk	Sporadically	0.13 (0.05)	0.013	0.09 (0.01–0.19)
	Regularly			0.22 (0.20–0.24)

were willing to collaborate and 24 answered the survey. Herd size median was 255 milking cows. The survey was about pen cleanliness and comfort, milking equipment, clinical mastitis (CM) management, milking routine and dry-off therapy. The greatest knowledge was about milking equipment. The least knowledge was on the aim of the dry therapy, although the level of application was 100%. Similarly, most of the producers informed an adequate CM management. Average incidence during the studied period was 21% (SD 8%). An extra pen for cows, regular pre-dipping and CM detection through foremilking were associated with low SM herd level (Table 1). Most producers applied good practices related to CM management, dry cow therapy and milking equipment, although deficient environment and milking routine were associated with higher SM incidence.

Key Words: somatic cell count, test day, survey

M91 Applying machine learning on feeding behavior data for estrus detection in dairy heifers. F. C. Cairo[1], L. G. R. Pereira*[2], M. M. Campos[2], T. R. Tomich[2], S. G. Coelho[3], C. F. A. Lage[3], A. P. Fonseca[3], A. M. Borges[3], and J. R. R. Dorea[4], [1]*Universidade Estadual do Sudoeste da Bahia*, *Itapetinga, BA, Brazil*, [2]*Brazilian Agricultural Research Corporation – Embrapa*, *Juiz de Fora, MG, Brazil*, [3]*Universidade Federal de Minas Gerais*, *Belo Horizonte, MG, Brazil*, [4]*University of Wisconsin-Madison*, *Madison, WI.*

The recent advances of sensor technology have allowed accurate estrus prediction using animal behavior information. The variables generated by Electronic feed and water bins have not been explored as predictive attributes for the development of models for estrus detection. The objectives of this study were (1) to quantify the effect of estrus on feed intake and animal behavior (feeding and drinking); and (2) to develop and evaluate predictive approaches to detect estrus using electronic feed and water bins data. Feed intake, animal behavior, and estrus events (n = 99) were measured in 57 Holstein × Gyr heifers (374 ± 21.2 kg). Previous to each estrus event, the total feed intake (as-fed basis), number of feed bins visits, number of water bins visits, time spent eating and time spent drinking water were computed. Three predictive approaches were evaluated: logistic regression (LR); artificial neural network (ANN); and random forest (RF). Twelve covariate sets were established to (ii.a) evaluate the prediction quality for estrus detection when long (0 to −174 h) or short (0 to −24 h) time series were used as predictors; (ii.b) to evaluate the ability of models to predict estrus 6 and 12 h in advance; and (ii.c) to evaluate the predictive quality for estrus detection when only feeding and drinking behavior data (without intake variables) were included as predictors. All variables obtained by electronic bins change on estrus day compared with previous days. All predictive approaches analyzed with and without the feed intake variable were accurate for estrus detection. The short time series (24h) before estrus is satisfactory for estrus detection. The prediction of estrus in advance with 6 and 12 h reduced the accuracy and stability of the models. ANN models, RF and LR showed an accuracy of over 80%, indicating the possibility of predicting estrus at 06 h in advance. The exclusion of feed intake data does not reduce the accuracy, sensitivity, and specificity of models for estrus detection, indicating the possibility of developing new sensor-based devices that allow estrus detection.

Key Words: artificial neural network, heat detection, precision livestock

M92 A field case study: Body condition change and metabolic status of transition cows in a small dairy farm. M. Rosales Gallardo*[1] and A. A. Barragan[2], [1]*The Pennsylvania State University, Penn State Extension*, *Lancaster, PA*, [2]*Department of Veterinary and Biomedical Sciences, Penn State University*, *University Park, PA.*

Feeding lactating ration leftovers to dry cows or partially using lactating rations to feed dry cows is a common practice often implemented by small-size dairy farmers. These practices could be associated, at least in part, to over conditioning of transition cows. The objective of this case study was to assess the effects of feeding lactating ration leftovers on body condition score (BCS) changes and β-hydroxybutyrate (BHB) concentrations during the transition period in dairy cows. This field case study was conducted in a 60-cow dairy farm in Lancaster County in Pennsylvania, which fed lactating rations to their dry cows during the case study length. Eleven dairy cows were enrolled at 35 ± 3 d before calving and followed for 63 ± 3 d after calving. BCS was recorded weekly by the same person during the study period. Blood samples for assessment of BHB were collected at 0 ± 3, 7 ± 3, 14 ± 3, 21 ± 3, 28 ± 3 and 35 ± 3 d after calving. The data were analyzed using the MIXED procedure of SAS. The average BCS of this group of cows at calving (0 ± 3 DIM) was 3.72 ± 0.16 pts. This condition rapidly decreased to 3.29 ± 0.15 pts. on d 7 ± 3 after calving and to 3.14 ± 0.14 pts. at d 14 ± 3 after calving. BCS reached the lowest point at 21 ± 3 d (3.04 ± 0.14 pts.) after calving. Even though a drop in BCS is expected after calving, the study animals lost condition in a rapid and exacerbated manner. The BHB concentration at calving was 0.99 ± 0.20 mmol/L and, only second lactation cows had higher BHB concentrations at 14 ± 3, 21 ± 3 DIM compared with their BHB concentration at calving. Furthermore, in this group of cows, there were 2 subclinical ketosis cases (i.e., BHB >1.2 mmol/L). The results from this field case study suggest that a common dry cow feeding practice in small farms may generate a rapid and exacerbated fat mobilization in the early lactation period, which in turns may increase the risk of infectious and metabolic diseases, impairing the welfare and performance of dairy cows.

Key Words: small farm, body condition, transition cows

M93 Immunoglobulin G and serum total protein concentration assessment in dairy calves over the first 2 weeks of age. A. Correa*, A. Villadecabres, and N. Silva-del-Río, *Veterinary Medicine Teaching and Research Centre, University of California-Davis*, *Tulare, CA.*

The aims of the present study were to: (1) evaluate changes of serum IgG (SIgG) and serum total protein (STP) concentration of dairy calves during the first 16 d of life, and (2) investigate if predictive models for SIgG concentration based on STP could be improved when adjusted by hematocrit (Hct). Enrolled calves (24 Jersey, 41 Holstein) arrived at a commercial raising operation from 2 source dairies. Prior arrival, calves were bottle fed 3 meals of pasteurized colostrum (2 L per meal) at (mean ± SD) 38 ± 26 min, 7 h 19 min ± 1 h 5 min, and 7 h 45 min ± 1h after birth. Concentration of SIgG (single radial immunodiffusion), STP (BRIX refractometer), and blood Hct (centrifugation), were determined in blood samples collected from calves immediately after arrival at the calf operation (1 d) and 4, 8, 12 and 16 d of life. The MIXED procedures of SAS were used to evaluate changes over time of SIgG and STP as well as the predictive value of STP corrected by Hct. At 1 d, SIgG ranged from 252 and 10,619 mg/dL (mean: 2,124 mg/dL), STP from 3.6 to 9.3 g/dL (mean: 6.0 g/dL) and Hct from 18 to 44% (mean: 30%). During the study period, on 1, 4, 8, 12 and 16 d, calves had SIgG <1,500 mg/dL (n = 11, 20, 28, 36 and 45 respectively) and serum TP <5.5 g/dL (n = 15, 26, 31, 47 and 63 respectively). Relative to 1 d, SIgG tended to decrease at 4 d (205 mg/dL), and it was significantly lower at 8 d (343 mg/dL), 12 d (583 mg/dL) and 16 d (747 mg/dL). Relative to 1 d, STP concentration significantly decrease with time at 4 d (0,344 g/dl), 8 d (0.470 g/dl), 12 d (0.704 g/dl) and 16 d (1.107 g/dl). There was a moderate correlation between SIgG at 1 d with STP at 1 d (r = 0.66), 4 d (r = 0.61, 8 d (r = 0.63), 12 d (r = 0.54), and 16 d (r = 0.66). The model fit for SIgG prediction did not improve when STP was adjusted by Hct. In summary, our results indicate that SIgG and STP declined during the first 2 weeks of life. Further studies should determine the specificity and sensitivity of SIgG and STP during the first 2 weeks of life as diagnostic tools for failure of passive transfer of colostrum.

Key Words: calf, immunoglobulin, total protein

M94 Preparation and evaluation of a film-forming teat dip containing chitosan for the prevention of mastitis. H. Zhang*, H. Jiang, Z. Han, Y. Mao, and Z. Yang, *College of Animal Science and*

Technology, Yangzhou University, Yangzhou, Jiangsu, PR China.

Postmilking teat dip is an effective procedure for prevention of mastitis in dairy cows during lactation. Film-forming teat dip was developed to improve the effectiveness of postmilking disinfection by leaving a germicidal film on the teats to against mastitis pathogens. Chitosan is a nontoxic and biodegradable polysaccharide that has received attention due to its great film-forming capacity and antibacterial effect. Aim of the trial was to evaluate the efficacy of a film-forming teat dip containing chitosan in preventing mastitis of dairy cows. In vitro antibacterial test, the film-forming teat dip agent (FFTDA), with 1.0% 50 kDa chitosan and 4% povidone iodine, showed better antibacterial effect against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus* than the agent with no chitosan ($P < 0.05$). Then the efficacy of FFTDA was compared with a conventional iodine postmilking teat dip on 48 healthy Chinese Holstein dairy cows. The right mammary gland half was assigned to receive FFTDA (treated group), the other side was dipped into conventional iodine bath (control group). Treatments were applied for 4 weeks, for 3 milkings per day. Milk samples were collected from each quarter at 0, 2, 4 weeks and analyzed for nutrients composition, somatic cell count (SCC) and somatic cell score (SCS). Subclinical mastitis was diagnosed by LMT test every week. After clinical trial of 4 weeks, there were no significant differences in milk fat content, protein content, lactose content, total solids content, SCC and SCS between treated group and control group. In the end of the trial, the incidence of subclinical mastitis teat of treated group and control group were 9.38% and14.58%, respectively. SCS was significantly lower than the beginning of the trial in the treated group ($P < 0.05$), while there was no significant difference in the control group. The results indicated that this film-forming teat dip containing chitosan can effectively reduce SCS, and it has a good effect on the control of subclinical mastitis in dairy cow.

Key Words: bovine mastitis, chitosan, film-forming teat dip

M95 Association of subclinical hypocalcemia at calving with productive outcomes in multiparous Jersey cows. A. Valldecabres* and N. Silva-del-Río, *Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA.*

The most appropriate blood calcium (Ca) threshold to define subclinical hypocalcemia (SCH) is still under study. Currently, there is a wide range of suggested definitions, and variability on its reported associations with productive outcomes, which have not been described in Jersey cows. Our aim was to evaluate the association of SCH at calving with subsequent lactation milk and energy-corrected milk (ECM) yield, fat% and protein%, in 609 multiparous Jersey cows from 2 commercial herds fed acidifying prepartum diet. Blood samples for total serum Ca concentration determination were collected from the coccygeal vessels at 3 h 10 min (±2 h 17 min) after calving. Monthly test milk yield, fat% and protein% information up to the 10th test was obtained from the Dairy Herd Improvement Association. Milk yield, ECM, fat% and protein% were analyzed with SAS (version 9.4); with multiple linear regression, using a 2-piece linear function for days in milk and cow as the subject in the repeated statement. Considered covariates for all outcomes were: parity, herd, previous lactation length and 305-d mature equivalent milk yield, dry period length, calving body condition and locomotion scores, calving easiness, oral Ca supplementation, and somatic cell linear score at test. Milk yield was also considered in the fat% and protein% models. Serum Ca thresholds among 1.80 and 2.20 mmol/L at 0.02 mmol/L intervals were used to define SCH. The Ca threshold significant for the type 3 test of fixed effects, and leading to the smallest P-value and most extreme β coefficient for the solution for fixed effects, was chosen to define SCH. Subclinical hypocalcemia (Ca ≤ 2.18 mmol/L) was associated with 1.52 and 1.88 kg/d more of milk and ECM, respectively ($P < 0.001$). For milk fat%, SCH (Ca ≤ 1.96 mmol/L) was associated with 0.12 units of milk fat%/d more ($P = 0.01$); and SCH (Ca ≤ 1.80 mmol/L), with 0.06 units of milk protein%/d less ($P = 0.03$). Similar effects were observed for additional thresholds evaluated. Further studies are needed to elucidate the applicability of a SCH definition.

Key Words: dairy cow, hypocalcemia, transition cow

M96 Switchgrass (*Panicum virgatum*) harvested using two different strategies in lactating dairy cow rations. B. Lemay*[1], R. Nagle[1], A. J. Carpenter[1], T. J. DeVries[1], P. H. Luimes[1], M. Thimmanagari[2], J. DeBruyn[2], and A. Heeg[2], [1]*University of Guelph, Guelph, ON, Canada*, [2]*OMAFRA, Ontario, Canada.*

Switchgrass (*Panicum virgatum*) is a native tall grass that has been researched as a novel species in the biomass industry and as a promising crop for livestock feed. Two harvesting strategies are employed for switchgrass hay: switchgrass is 1) cut and baled in the fall, or 2) left to over-winter in the field, and cut and baled in the spring. This study aimed to compare switchgrass harvested by either of these 2 methods to use of wheat straw in lactating dairy cow rations. Holstein cows (n = 12, DIM = 158 ± 48.4; parity = 1.6 ± 0.67) were exposed to each of 3 treatments in a 3 × 3 LSD with 14-d periods (10d of adaptation and 4d of sampling). Treatments consisted of TMR containing equal amounts of: 1) wheat straw (CON), 2) spring-baled switchgrass (SSG), or 3) fall-baled switchgrass (FSG) at 0.5% inclusion, on a DM basis. Milk samples were collected twice daily at each milking, and feed and blood samples were collected before the morning feeding. Feed refusal samples were taken and subjected to particle size analysis to determine feed sorting. All statistical analyses were conducted using a repeated measures mixed-effect linear regression model with the fixed effect of treatment and random effects of cow and day. Analysis of feed samples indicated no differences between treatments for NDF, ADF, CP, or TDN ($P \geq 0.60$). DMI was consistent between treatments (SSG = 23.5, FSG = 24.8, CON = 24.1 kg/d; SE = 1.35; $P \geq 0.63$), as was milk yield (FSG = 27.6, SSG = 28.0, CON = 27.8.7 kg/d; SE = 1.37; $P \geq 0.93$). Milk fat was greater (SE = 0.015; $P = 0.01$) for cows fed a TMR containing CON (4.5%) vs. SSG (4.2%), but neither treatment differed from FSG (4.4%; $P \geq 0.23$). Milk protein was greater for cows fed SSG (3.44%) or CON (3.44%) compared with cows fed FSG (3.40%; SE = 0.05; $P = 0.01$), but did not differ between CON and SSG ($P = 0.99$). Blood metabolites (BHBA, NEFA, and glucose) did not differ between treatments ($P \geq 0.32$). No differences in sorting of the treatment TMRs were observed ($P \geq 0.18$). Results suggest that feeding switchgrass can successfully replace wheat straw in lactating rations and that switchgrass harvested strategy may influence milk components in dairy cows.

Key Words: switchgrass, milk production, harvest strategy

M97 Simultaneous minimization of diet costs and phosphorus excretion on dairy farms. A. F. White* and L. E. Moraes, *The Ohio State University, Columbus, OH.*

The study objective was to develop a mathematical programming model capable of simultaneously optimizing diets with different weights for least cost (LC) and least on farm P-balance (LP). The ration ingredients, herd composition, and animal categories were designed to represent the Jersey herd of the Ohio State University Waterman Dairy Center. The model constraints were set to meet the current recommendations of the NRC (2001), and additional constraints based on industry recommended practices were specified to limit the inclusion of specific feeds in the diet. Using OpenSolver (v.2.9.0, opensolver.org), 3 objective functions were individually optimized to (1) minimize the diet cost; (2) minimize the excess of P balance in the system, computed as the difference between P excreted and P uptake by forage production on-farm; and (3) minimize the weighted deviations from the values of the 2 previously optimized objective functions. A grid of 100 potential weights were used that varied the size of the effect of each deviation from the given optimization model on the current optimized objective. The LC and LP scenarios had whole farm feed costs of $2,285 and $2,719 (19% increase with respect to the LC diet), respectively; the farm P balance was 10.1 kg/d and 3.3

kg/d (67% decrease). Thus, at the extreme points of the weight grid, a substantial decrease in P balance was obtained; however, at a substantial increase in diet costs. The trade-offs between the 2 goals set by the weighting scheme, however, allowed the identification of a set of diets that all met the NRC (2001) requirements while having different costs and determining different P balances on the farm. For example, the weighted goal programming model identified a diet that allowed a 37% reduction of P balance occurring with a 0.4% increase in cost (with respect to LC). These initial reductions in P were generally accompanied by increased forage fed, increase of on-farm corn silage production, and increased purchase of hay. These optimizations suggest a potential use of weighted goal programming as a technique to identify diets that allow a reduction in on-farm P balance with limited effect on whole-farm feed cost.

M98 Effects of heat stress on rumination activity and ruminal in situ degradability in dairy cows. G. G. Maia[1], L. G. B. Siqueira[2], C. O. P. Vasconcelos[1], T. R. Tomich[2], L. S. A. Camargo[2], J. P. P. Rodrigues[3], R. A. Menezes[4], L. C. Gonçalves[4], B. F. Teixeira[5], R. O. Grando[5], L. A. G. Nogueira[1], and L. G. R. Pereira*[2], [1]*Universidade Federal Fluminense*, *Niterói, RJ, Brazil*, [2]*Empresa Brasileira de Pesquisa Agropecuária - Embrapa*, *Juiz de Fora, MG, Brazil*, [3]*Universidade Federal do Sul e Sudeste do Pará*, *Xinguara, PA, Brazil*, [4]*Universidade Federal de Minas*, *Belo Horizonte, MG, Brazil*, [5]*Instituto de Desenvolvimento do Alto Uruguai*, *Getúlio Vargas, RS, Brazil*.

The objective of this study was to evaluate the effects of heat stress (HS) on (1) time and daily pattern of rumination, and (2) in situ degradability in Holstein-Gyr crossbred dry cows. Nine cows, weighing 556.2 ± 97.1 kg were housed in a climate chamber (average THI = 83.1) or a freestall barn (average THI = 66.5) randomly assigned to treatments in a crossover design. For each animal, there were 25 d of data collection in each treatment. Rumination and intake patterns at different days or 2-h periods were evaluated using the MIXED procedure. Cows were fed corn silage ad libitum and 1 kg of concentrate as a maintenance diet. To measure rumination, we used a sensor attached to a collar. The in situ ruminal degradability was evaluated by incubation of concentrate, silage, and diet samples into the rumen of one fistulated cow for 6, 24, 48 and 96 h when this cow was under HS (climate chamber) and at the freestall (control) after 21 d of the starting of the treatment. The ruminal degradability kinetics were estimated using a uni-compartmental model with the Marquardt algorithm to fit nonlinear regression. The RT was affected by HS ($P < 0.001$) and time of the day ($P < 0.001$). The rumination pattern for control and HS cows had a first peak at approximately 04:00h and the magnitude of this peak in HS was 22.9% less than controls. The second RT peak occurred in the evening (~20:00h), when RT in HS was 27.8% less compared with controls. We observed a reduction in dry matter digestibility (DMD) of concentrate (85.1% vs 77.8%, control and HS, respectively; $P < 0{,}05$) and also in the slow-degradable fraction of concentrate (93.9% vs 85.1%, respectively; $P < 0{,}05$). Finally, DMD of the diet reduced from 73% to 70% for animals exposed to HS compared with controls. In conclusion, HS negatively affected the rumination time and in situ ruminal degradability in dry dairy cows.

Key Words: climate chamber, precision dairy farming, sensors

M99 Differences in total mixed ration dry matter percentage affect the feed intake and the milk yield of Holstein cows. V. S. Izquierdo*, C. Pizoni, J. P. Noschang, M. F. B. Savela, E. G. Malaguez, J. Halfen, E. Schmitt, B. S. Menezes, E. G. Xavier, A. A. Barbosa, C. C. Brauner, J. O. Feijó, V. R. Rabassa, F. A. B. Del Pino, M. N. Corrêa, *Núcleo de Pesquisa, Ensino e Extensão em Pecuária (NUPEEC), Departamento de Clínicas Veterinária, Universidade Federal de Pelotas*, *Pelotas, Rio Grande do Sul, Brazil*.

The high yielding cows need a balanced diet to keep the equilibrium between feed intake and productivity, any difference in diet qualities can impact directly the livestock's production, mainly on confinement systems. The aim of this study was to evaluate if the changes in the percentage of dry matter could negatively affect the feed intake and milk yield of Holstein cows. This study was performed on a commercial dairy farm in the south of Brazil, during the months of May to November of 2019. All the cows enrolled in this study were multiparous, housed in a compost barn and belonging from the highest milk production group (n = 120). For this study was used the database registered for a subset of cows during the period of 66 d totaling 617 observations of DMI from feeders that measure the intake. For the milk yield database was used the overall mean of milk production of the same group of cows during the same period. To measure the dry matter percentage, samples of TMR was collected and classified as ideal (between 45% and 60%, n = 45) and not ideal (<45% or > 60%, n = 21). The diet composition was based in water (15.87%), ground corn (1.98%), soybean meal (5.75%), soybean residue (5.16%), rice bran (3.77%), wet maize silage (3.97%), corn silage (53.56%), rice residue (1.98%) and pre-dried ryegrass (3.97%). The data were analyzed using the ANOVA one-way of the SAS program. The DMI was greater in the ideal group ($P < 0.01$) when compared with the not ideal group (19.77 ± 6.00 kg vs. 16.63 ± 7.47 kg of DMI). The milk yield showed a trend ($P = 0.07$) to be higher on the ideal group when compared with the not ideal (36.99 ± 3.06 vs. 35.48 ± 3.30 L/milk/day). These results suggest that cows feeding not ideal concentration of dry matter may reduce the DMI and tend to be less productive than those with ideal concentration.

Key Words: compost barn, intake measurement, dairy cows

M100 Effects of dietary organic acid and plant botanical supplementation on growth performance in Holstein calves challenged by heat stress. A. B. P. Fontoura*[1], V. Sáinz de la Maza-Escolà[1,2], B. N. Tate[1], J. T. Siegel Nieves[1], A. T. Richards[1], F. Wang[1,3], L. F. Wang[1,4], M. E. Van Amburgh[1], E. Grilli[2,5], and J. W. McFadden[1], [1]*Cornell University*, *Ithaca, NY*, [2]*University of Bologna*, *Bologna, Italy*, [3]*China Agricultural University*, *Beijing, China*, [4]*Henan Agricultural University*, *Zhengzhou, China*, [5]*VetAgro S.p.A*, *Reggio Emilia, Italy*.

Our objectives were to evaluate the effects of heat stress (HS) and dietary organic acid and plant botanical (OA/PB) supplementation on growth in calves. In a completely randomized design, 62 bull and heifer calves were assigned to 1 of 5 groups (n = 12–13/group): thermoneutral conditions (TN-Con), HS conditions (HS-Con), thermoneutral conditions pair-fed to HS-Con (TN-PF), HS with low-dose OA/PB (75 mg/kg of BW; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; AviPlus R; Vetagro, Italy; HS-Low), or HS with high-dose OA/PB (150 mg/kg of BW; AviPlus R; HS-High). Supplements were delivered as a twice daily bolus via the esophagus wk 1 through 13 of life; all calves received boluses equivalent for triglyceride. Post weaning, calves (62 ± 2 d; 91 ± 10.9 kg) remained in thermoneutral conditions (temperature-humidity index [THI]: 60 to 69) for a 7-d covariate period. Thereafter, calves remained in TN conditions or were moved to HS conditions (THI: 75 to 83) for 19 d. Clinical assessments and BW were recorded, and blood was sampled. Organs from HS-Con and TN-Con were harvested at trial completion. The mixed model included fixed effects of BW at birth, treatment, time, and their interaction. Rectal and skin temperatures, and respiration rates were greater in HS-Con, relative to TN-Con ($P < 0.01$). Dry matter intake (DMI) and average daily gain (ADG) were lower in HS-Con, relative to TN-Con ($P < 0.01$). Comparing HS-Con and PF-Con, ADG and gain:feed were similar. Plasma fatty acids were elevated in TN-PF versus all other groups ($P = 0.04$; not observed for HS-Con). Liver and small intestine weights were lower in HS-Con, relative to TN-Con ($P = 0.03$ and 0.15, respectively). DMI was greater with HS-Low, relative to HS-Con ($P < 0.01$). ADG for HS-Low and HS-High were not different from HS-Con or TN-Con (i.e., effect was intermediate). Compared with HS-Con, calves fed OA/PB tended have greater gain:feed ($P = 0.08$). We conclude that reductions in DMI account for losses in growth during HS and dietary OA/PB supplementation enhances HS resilience in calves.

Key Words: calf, heat stress, organic acid

M101 Effect of rearing conditions of primiparous cow prior to weaning on their performance and behavior after calving.

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The objective was to find whether cow growth, milk performance, and behavior are affected by their rearing to weaning in 84 d. Thirty-five Holstein heifers were assigned to 1 of 3 treatments: SM, n = 13, pen with mother (milked from d 2) to d 21, suck a mother's udder 3 times per d, then group pen (6 kg milk per d, bucket with nipple); SN, n = 9, after 3 d with own mother in pen with nursing cow, calves could drink at any time, usually 3–5 times per d, number of calves per nursing cow determined according to milk yield (6 kg milk per d and calf); H, n = 13, after having nursed their dams in individual pen for 24 h in hutches from d 2 to 56 (bucket with nipple, milk replacer, 6 kg per d), then loose housing pen to weaning (bucket with nipple, milk replacer, 6 kg per d). Group SM allowed 21 d suckle, 63 d bucket fed; SN allowed 84 d suckle; H allowed 1 d suckle, 83 d bucket fed. All calves were weaned at d 84. After weaning from milk feeding, all heifers were kept in age-balanced groups in bedded pens with the same ration to the calving. During the lactation, live body weight (LBW) was measured each month and milk yield (MY) each d. Cows were milked in the parlor from d 4. Learning was evaluated at the 5th month. Cows solved 6 tasks during 3 d. The data were analyzed using a General Linear Model ANOVA. At the 30th d, the LBW tended to be the highest in SN (SM 528 ± 11 kg, SN 572 ± 15 kg, H 533 ± 12 kg). On the lactation end, the highest LBW was in SN and the lowest in H (SN 623 ± 16 kg, SM 613 ± 12 kg, H 570 ± 13 kg, $P < 0.05$). The SN tended to the highest production of milk (SM 7144 ± 241 kg, SN 7345 ± 319 kg, H 7147 ± 234 kg), and the SM for FCM (SM 6521 ± 203 kg, SN 6278 ± 268 kg, H 6340 ± 197 kg) for 305-d lactation. Group SN crossed the maze fastest (SM 1141 ± 120 s, SN 810 ± 160 s, H 1121 ± 119 s). The number of vocalizations differed significantly (SM 32 ± 6, SN 21 ± 4, H 10 ± 3, $P < 0.01$). The results indicated that the rearing method to weaning may have an impact on dairy cow performance and behavior.

Key Words: rearing, growth, milk

M102 Relationships between longissimus dorsi muscle depth, body weight, and parity during the first five months of lactation.

C. J. McCabe* and J. P. Boerman, *Purdue University, Department of Animal Sciences*, *West Lafayette, IN*.

Due to insufficient dry matter intake and heightened nutrient demands in the first weeks of lactation, cows mobilize their own muscle and adipose tissue to bridge these nutrient gaps. The objective of this study was to analyze changes in muscle depth and BW in primiparous and multiparous animals from early through mid-lactation. Holstein dairy cows (n = 38; 1st lactation n = 14, 2nd+ lactation n = 24) were weighed and their muscle depth was measured 1x per month following the morning milking for 5 mo to quantify BW and muscle depth changes from early through mid-lactation. We captured muscle depth images from the right side of each cow from the 12th intercostal space of the longissimus dorsi muscle using an ALOKA SSD-500 ultrasound with a linear transducer. At each time point, 3 images were captured, and an average was quantified using ImageJ software (NIH; Bethesda, MD). There were no differences in longissimus dorsi muscle depth between primiparous and multiparous cows ($P = 0.77$). However, BW was higher for multiparous dairy cows (577 vs. 691 kg; $P < 0.0001$) compared with primiparous cows. Muscle depth was lower for the second month of lactation (4.07 cm) compared with the fourth month of lactation (4.42 cm; $P < 0.05$). Measurements taken over the study for muscle depth and BW were positively correlated ($r = 0.35$; $P < 0.0001$). Change in BW and change in muscle depth in the first month were positively correlated ($r = 0.83$; $P < 0.0001$). Extent of both BW and muscle depth loss in the first month was negatively correlated with BW change in the second month ($r = -0.66$ and $r = -0.59$; $P < 0.05$, respectively). Likewise, muscle depth change from in the second month of lactation was negatively correlated with muscle depth change in the third month ($r = -0.88$; $P < 0.0003$). A considerable amount of BW, specifically muscle, is mobilized during the first 2 mo of lactation with more accretion of muscle starting in the third month of lactation. Understanding the timing and extent of muscle mobilization and accretion will allow us to meet nutrient requirements of lactating dairy cows.

Key Words: ultrasound, muscle depth, parity

Ruminant Nutrition: Calves and Heifers

M103 Supplement protein level affects performance and reproduction of grazing heifers. A. F. Machado[1], V. C. L. Moraes[1], D. L. Souza Netto[1], P. V. F. Correa[1], S. E. F. Guimaraes[1], G. M. Santos[2], and M. I. Marcondes*[1], [1]*Universidade Federal de Vicosa*, *Vicosa, MG, Brazil*, [2]*Univicosa*, *Vicosa, MG, Brazil*.

Evaluations of the interaction between nutrition and reproduction of dairy heifers grazing intensively managed pasture are scarce. Thus, we aimed to evaluate performance, muscle development, oocyte quality and in vitro embryo production of grazing crossbred heifers fed increasing CP levels in the supplement. Eighteen pubertal crossbred heifers (Holstein x Gyr; initial BW of 350 ± 8.0 kg) were used in a 90-d trial. Two supplements (12 and 24% CP, S12CP and S24CP) and control treatment (mineral mixture, MM) were randomly assigned to the heifers. Three ovarium pic-up were performed. DMI and CP intake were greater for supplemented (SUP) compared with MM. SUP heifers had greater ADG ($P = 0.003$) and rib eye area ($P = 0.04$) than MM, and carcass ultrasonography indicated a trend for greater rib eye area for S24CP comparing with S12CP (Table 1). Viable oocytes were not affected by supplementation strategy. However, cleavage and blastocyst rates were negatively affected by SUP ($P < 0.05$), and S12CP had lower blastocyst rate than S24CP ($P = 0.012$; Table 1). In summary, supplementation with concentrate containing 24% CP might be an appropriate strategy for satisfactory performance during the rainy season in tropical climates. In contrast, the in vitro embryo production was impaired with supplementation.

Key Words: supplementation, oocyte quality, embryo production

M104 Performance of calves fed starter and grower diets formulated with inorganic, organic, or organic trace minerals with additives from birth to 6 months of age. D. Ziegler[1], H. Chester-Jones[1], B. Ziegler[2], A. Manthey[2], E. Dufour*[2], and K. Mjoun[3], [1]*University of Minnesota*, *Waseca, MN*, [2]*Hubbard Feeds*, *Mankato, MN*, [3]*Alltech*, *Nicholasville, KY*.

One-hundred six (2 to 5 d old) Holstein heifer calves (40.0 ± 0.57 kg) from 2 commercial dairies were randomly assigned to 1 of 3 calf starter (CS) treatments formulated with different trace minerals (TM) and additives to evaluate performance and health during the 56-d nursery phase (NP) and the 112-d grower phase (GP). All calves were fed a non-medicated 20% CP: 20% Fat milk replacer (all-milk protein) fed at 0.28 kg in 2 L of water 2× daily from d 1 to 35 and 1x daily from d 36 to weaning at d 42. Calves were fed a medicated (Decoquinate at 45.4 g/ton) 18% CP texturized CS free choice in the NP. Treatments were as follows: 1) CS formulated with inorganic TM (INORG); 2) CS formulated with organic TM (ORG); 3) CS formulated with organic TM and an additive containing yeast products, DFM's and other additives (ORGAD). In the GP, calves were fed by treatment in group pens and offered 2.73 kg of grain mix medicated with Monensin at 39 g/ton supplemented as in NP and fed hay free choice daily. Data were analyzed using the PROC mixed procedure of SAS and repeated measures analyses applied where appropriate. There was a trend ($P = 0.08$) for increased ADG in the NP for calves fed ORGAD compared with ORG with INORG being intermediate, averaging 0.70, 0.64 and 0.66 kg/d, respectively. Total CS intake in NP was lower ($P < 0.05$) for calves fed ORG compared with INORG and ORGAD averaging 44.7, 50.0 and 51.6 kg, respectively. Health costs trended higher ($P = 0.09$) in the NP for calves fed INORG compared with ORG and ORGAD averaging 0.75, 0.22 and 0.17 \$/d, respectively. In the GP, there was a trend ($P = 0.07$) for increased ADG for calves fed ORGAD compared with calves fed INORG with ORG being intermediate averaging 1.02, 0.98 and 0.99 kg/d, respectively. In the GP there was no difference ($P > 0.05$) in total DMI/d or gain:feed averaging 3.91 kg/d and 0.27, respectively. Under conditions of this study there was no measured benefit to feeding an organic compared with an inorganic TM in the NP or GP. The addition of additives to an organic TM mix may improve calf health and performance.

Key Words: calf performance, trace minerals, calf starter

M105 Use of sensory additives to mask bitter taste in calf milk replacers. M. Terré*[1], M. Verdú[2], A. Frongia[2], R. Cresci[2], and M. Blanch[3], [1]*IRTA*, *Caldes de Montbui, Spain*, [2]*bonÀrea Agrupa*, *Guissona, Spain*, [3]*Lucta S.A*, *Bellaterra, Spain*.

There are several feed additives used for dairy calves that cause palatability problems. A model to cause milk replacer (MR) aversion to dairy calves was developed. The model consisted on adding 30 g/kg of a mix of commercial products with bitter taste (Bittermix) to the MR, and animals who received Bittermix (BM) had more MR refusals, and a decrease in the MR intake rate. To evaluate the potential of 2 sensory additives differing in the sweet fraction to mask BM aversion in the MR, 37 Holstein male calves (6.5 ± 0.93 d of age and 40.2 ± 1.40 kg of BW) were raised under the same conditions following a common MR (25.3% CP, 21.1% fat) feeding program. When calves (38.5 ± 1.12 d of age and 57.7 ± 1.70 kg of BW)were able to consume 8 L/d at 12.5% DM concentration in 2 feedings, the aversion test was performed adding one of the following products to the MR: no supplementation (CTRL; n = 9); BM at the dose of 30 g of BM/kg of MR; (BM; n = 9); BM plus sensory additive 1 at

Table 1 (Abstr. M103). Performance and reproductive parameters of Holstein × Gyr heifers not supplemented (MM) or supplemented with concentrate containing 12% CP (S12CP) or 24% CP (S24CP) in a rotational grazing system

		Supplement			*P*-value[1]		
Item	MM	S12CP	S24CP	SEM	MM×SUP	S12CP× S24CP	PER
DMI, kg/d	7.36	8.04	8.58	0.307	0.014	0.220	0.091
CP intake, kg/d	1.28	1.30	1.63	0.053	0.008	0.001	0.327
ADG, kg/d	0.39	0.60	0.69	0.059	0.003	0.381	—
Ribeye area, cm^2	53.32	56.00	61.56	1.983	0.040	0.070	—
Viable oocytes, no	6.07	5.39	7.25	1.572	0.926	0.420	0.007
Cleavage rate, %	41.17	23.92	36.71	5.716	0.017	0.102	0.676
Blastocyst rate, %	27.91	3.02	17.23	3.924	0.001	0.012	0.816

[1]SUP×MM = supplementation effect; S12CP×S24CP = effect of supplement protein level; PER = period effect.We observed no TR×PER interaction ($P > 0.05$).

the dose of 2 g/kg of MR (SA1; n = 10); and BM plus sensory additive 2 at the dose of 2 g/kg of MR (SA2; n = 9). The aversion test lasted 7 d, and MR intake and time devoted to consume the MR were recorded at each feeding throughout the aversion week. Data were analyzed with a mixed-effects model accounting the fixed effects of MR supplementation, feeding during the aversion week, and their interaction, and calf as random effect. The incidence of MR refusals was analyzed with a generalized mixed model considering treatment as fixed effect. The incidence of refusals and time devoted to consume the MR were similar in all 4 treatments. However, the eating rate of calves supplemented with SA2 was similar to CTRL calves, in contrast to calves supplemented with SA1 that had similar eating rate to BM calves. Sensory additive 2was able to revert the aversion effects of BM exhibiting a similar MR eating rate than CTRL calves.

Key Words: aversion test, calf, sensory additive

M106 Effects of a blend of essential oils in milk replacer on blood parameters of dairy heifers.

J. P. Campolina[1], S. G. Coelho[1], A. L. Belli[1], L. G. R. Pereira[2], T. R. Tomich[2], W. A. Carvalho[2], and M. M. Campos*[2], [1]*Department of Animal Science, Veterinary School, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil*, [2]*Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA), National Center for Research on Dairy Cattle, Juiz de Fora, MG, Brazil.*

The rise of organic dairy farms increased demands for natural alternatives to improve animals' development and health. The objective of this study was to characterize the hematological and biochemical responses of dairy heifers supplemented with of a commercial blend of essential oils (Apex calf, Adisseo, China) in milk replacer (MR) and its carryover effects for 30 d after weaning. Twenty-nine newborn Holstein x Gyr crossbred dairy heifers were randomly distributed among 2 treatments: Control (CON), no essential oils, n = 15 and blend of essential oil added to milk replacer (MR) (BEO), 1 g/calf/d, n = 14. Heifers received 5 L of MR with 15% of total solids, divided in 2 daily meals until 60 d of age, when they were weaned. Water and calf starter were offered in buckets for ad libitum intake. Jugular blood samples were collected every 7 d, 3 h after morning feeding, for β-hydroxybutyrate (BHB), urea and glucose concentrations, every 14 d for IGF-1 concentrations, and on d 0, 30 and 60 for erythrogram and leukogram analysis. Data were analyzed using R (R Core Team, 2019), as a randomized block model with repeated measures, using the linear mixed model. All blood metabolites were not altered by BEO supplementation. Urea and BHB concentrations increased with age ($P \leq 0.05$). IGF-1 concentrations increased with age on the preweaning phase ($P \leq 0.05$). Glucose did not change during preweaning period. These changes are directly correlated gut and calf development. There were no differences in erythrogram parameters between BEO and CON. Leukogram parameters showed decreased counts of basophil and platelet cells in BEO treatment ($P \leq 0.05$). An interesting interaction effect was found also for lymphocytes, where values of d 30 and 60 were different from d 1. The lower counts of basophil and platelets on BEO treatment may influence and modulate inflammatory response by secretion of immune modulators, growth factors or chemotaxis on variety of white blood cells. Feeding BEO to preweaned heifers on MR did not affect blood metabolites or IGF-1 concentration. However, it showed signs of immunity improvement.

Key Words: blood metabolites, erythrogram, health

M107 Effects of a blend of essential oils in milk replacer on gut weight and histology of dairy calves.

J. P. Campolina[1], S. G. Coelho[1], A. L. Belli[1], F. S. Machado[2], L. G. R. Pereira[2], T. R. Tomich[2], S. F. Costa[3], and M. M. Campos*[2], [1]*Department of Animal Science, Veterinary School, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil*, [2]*Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA), National Center for Research on Dairy Cattle, Juiz de Fora, MG, Brazil*, [3]*Department of Veterinary Medicine, Federal University of Lavras, Lavras, MG, Brazil.*

Calves are born functionally non-ruminant. Therefore, the use of additives that helps its digestive system and other organs development are used in dairy farm to optimize the preweaning phase. The objective of this study was to characterize changes on the gut development of calves supplemented with of a commercial blend of essential oils (Apex calf, Adisseo, China) on milk replacer. Sixteen newborn Holstein x Gyr crossbred bull calves were randomly distributed among 2 treatments: Control (CON), no essential oils, n = 8; and blend of essential oil added to milk replacer (MR) (BEO), 1 g/calf/d, n = 8. They received 5 L of MR with 15% of total solids, divided in 2 daily meals until 60 ± 1 d, when they were euthanized to compare internal organs development. Water and calf starter were offered in buckets for ad libitum intake. Weights of spleen, bladder, liver, pancreas, kidney, rumen-reticulum, omasum, abomasum, small and large intestine, tongue, heart and respiratory tract were compared with percentage of empty BW. For comparative histology samples were collected from rumen ventral sac, rumen dorsal sac, omasum, abomasum, duodenum, ileum and colon. For each sample papilla`s or villi area, height and mitotic index (MI) of epithelium basal layer were analyzed. For MI determination, 2000 cells of the basal layer were counted using a light microscope. Estimation considered the ration between number of cells in mitotic division and total counted cell number. Data were analyzed using R (R Core Team, 2019), as a randomized block model with repeated measures, using the linear mixed model. Most of the organs compared presented similar weights among treatments. However, differences were found for pancreas, respiratory tract and small intestine, were BEO calves presented heavier organs ($P \leq 0.05$). For cellular development BEO calves presented a higher ilium villi height ($P \leq 0.05$). Since internal organs have a higher metabolic rate, and responds to alterations in feeding patterns, the BEO could be an option for increasing gut development during the preweaning period.

Key Words: gut development, preweaning phase, rumen-reticulum

M108 Pre- and postweaning performance of calves fed milk replacer formulated with different levels of sodium butyrate.

D. Ziegler[1], H. Chester-Jones[2], B. Ziegler[2], A. Manthey*[2], E. Dufour[2], and K. Mjoun[3], [1]*University of Minnesota, Waseca, MN*, [2]*Hubbard Feeds, Mankato, MN*, [3]*Alltech, Nicholasville, KY.*

One-hundred eight (2 to 5 d old) Holstein heifer calves (39.1 ± 0.69 kg) from 2 commercial dairies were randomly assigned to 1 of 4 milk replacer (MR) treatments formulated with different levels of Viligen, a blend of short chain fatty acids and prebiotic components, sodium butyrate (SB) to evaluate pre- (d 1 to 42) and post-weaning (d 43 to 56) calf performance and health. The study was conducted between August and December, 2018. All calves were fed a non-medicated 20% CP:20% Fat milk replacer (all-milk protein) fed at 0.28 kg in 2 L of water 2× daily from d 1 to 35 and 1× daily from d 36 to weaning at d 42; supplemented (d 1 to 14) with neomycin sulfate and oxytetracycline at 22 mg/kg BW/d. All calves were fed a medicated (Decoquinate at 45.4 g/ton) texturized calf starter (CS; 18% CP as fed). Milk replacer treatments were as follows: 1) MR without added SB, (CON); 2) MR formulated with 0.24% SB, (SB24); 3) MR formulated with 0.48% SB, (SB48); and 4) MR formulated with 0.72% SB, (SB72). Water and CS were offered free choice from d 1 to 56. Data were analyzed using the PROC mixed procedure of SAS and repeated measures analyses applied where appropriate. There were no differences ($P > 0.05$) in preweaning, postweaning, or total gains (d 1 to 56) averaging 0.56, 1.12 and 0.70 kg/d, respectively. There was a trend ($P = 0.08$) of a linear decrease in overall (d 1 to 56) hip height and hip height gain with increasing levels of sodium butyrate fed in the MR. Pre- and post-weaning CS intake was similar ($P > 0.05$) across treatments averaging 18.1 and 31.2 kg, respectively. There were no differences ($P > 0.05$) in preweaning, postweaning or overall (d 1 to 56) feed efficiency across treatments averaging 0.61, 0.51 and 0.56, respectively. There were no differences in overall (d 1 to 56) health costs or fecal scores (1 to 4; where 1 = normal, 4 = watery), across treatments, averaging 90 cents and 1.27. Under conditions of this study calves fed a MR formulated with different levels of SB did not affect ADG, gain:feed or health of calves pre- or postweaning.

Key Words: calf performance, sodium butyrate, milk replacer

M109 Performance and health of dairy calves fed milk replacers formulated with different levels of coconut oil as a partial replacement of animal fat in two feeding rates. D. Ziegler*[1], H. Chester-Jones[1], B. Ziegler[2], A. Manthey[2], and E. Dufour[2], [1]*University of Minnesota*, *Waseca, MN*, [2]*Hubbard Feeds*, *Mankato, MN*.

One-hundred six (2 to 5 d old) Holstein heifer calves (39.7 ± 0.63 kg) from 2 commercial dairies were randomly assigned to 1 of 5 milk replacer (MR) treatments, formulated with animal fat (AF) or a blend of coconut oil (CO) and AF (FB) to evaluate calf performance and health. The study was conducted between November 2018 and February, 2019. All calves were fed a medicated (Decoquinate at 45.4 g/ton) texturized calf starter (CS; 18% CP as fed). Water and CS were offered free choice from d 1 to 56. All MR were formulated with all-milk protein and Bio-Mos. All calves were supplemented (d 1 to 14) with neomycin sulfate and oxytetracycline at 22 mg/kg BW/d. Treatments were as follows: 1) 24% CP:20% Fat MR, (AF) fed at 0.28 kg in 2.00 L of water 2× daily from d 1 to 35 and 1× daily from d 36 to weaning at d 42 (AFL); 2) 24% CP:24% Fat MR formulated with a FB of 85% AF,15% CO; fed as in TRT 1, (LCOL); 3) 24% CP:24% Fat MR formulated with a FB of 75% AF, 25% CO fed as in TRT 1, (HCOL); 4) MR as in TRT 2, fed at 0.43 kg in 2.98 L of water 2× daily from d 1 to 42 and 1× daily from d 43 to weaning at d 49, (LCOH); 5) MR as in TRT 3, fed as in TRT 4; (HCOH). Data were analyzed using the PROC mixed procedure of SAS and repeated measures analyses applied where appropriate. There were no differences in ADG ($P > 0.05$) d 1 to 56, d 57 to 84 or d 1 to 84 averaging 0.79, 0.90 and 0.83 kg/d, respectively. Hip height gain d 1 to 56 was greater ($P < 0.05$) for high compared with low MR feeding rates averaging 13.10 and 11.75 cm, respectively. Total (d 1 to 56) CS intake, DMI and gain:feed ratio was higher ($P < 0.05$) for AFL, LCOL and HCOL compared with LCOH and HCOH averaging 56.8, 77.9 kg, 0.58 and 34.8, 71.8, 0.65 respectively. There were no differences in health costs or scouring days (1 to 4; where 1 = normal, 4 = watery), d 1 to 56 averaging 50 cents and 1.82 d, respectively. Under conditions of this study there was no difference in performance or calf health when feeding a 24% CP:24% Fat MR formulated with 2 different levels of CO.

Key Words: calf performance, milk replacer, animal fat

M110 Pre- and postweaning performance and health of dairy calves fed milk replacers supplemented with a direct-fed microbial and/or brewery yeast cell wall. D. Ziegler*[1], H. Chester-Jones[1], and T. Marubashi[2], [1]*University of Minnesota*, *Waseca, MN*, [2]*Calpis America Inc*, *Peachtree City, GA*.

One-hundred seven (2 to 5 d old) individually fed Holstein heifer calves (39.9 ± 0.66 kg) from 2 commercial dairies were randomly assigned to 1 of 4 milk replacer treatments (MR) supplemented with an organic direct-fed microbial (DFM) and/or brewery yeast cell wall (BYCW) to evaluate pre- (d 1 to 42) and postweaning (d 43 to 56) calf performance and health. The study was conducted between April and July, 2019. Treatments included: 1) all-milk protein, non-medicated MR 20% CP:20% Fat fed at 0.28 kg in 2 L of water 2× daily from d 1 to d 35 and 1× daily from d 36 to weaning at d 42, (CON); 2) MR as in CON supplemented with (BYCW) at a rate of 7.5 g daily, (BYCW); 3) MR as in CON supplemented with 5 g daily of a DFM containing *Bacillus subtilis* strain C-3102 (3.0×10^9 cfu/calf, d 1 to 42 ; DFM); 4) MR as in CON supplemented with 5 g daily of DFM and 7.5 g daily of BYCW, (DFMBYCW). All calves were offered a (18% CP as fed) medicated calf starter (Decoquinate at 45.4 g/ton) and water free choice from d 1 to 56. Data were analyzed using the PROC mixed procedure of SAS and repeated measures analyses applied where appropriate. There were no differences ($P > 0.05$) in pre- (d 1 to 42), post weaning (d 43 to 56), or overall (d 1 to 56) gains averaging 0.56, 1.07, 0.68 kg/d, respectively. Pre- (d 1 to 42) and post weaning (d 43 to 56) calf starter intake was similar across treatments averaging 18.1 and 31.6 kg total intake, respectively. There was a difference ($P < 0.05$) in number of days fecal score = 4 (d 1 to 42; where 1 = normal, 4 = watery), for calves fed CON averaging 0.58 d compared with 0.18 and 0.22 d respectively for BYCW and DFMBYCM with DFM being intermediate at 0.35 d. Health costs were similar across treatments. Under conditions of this study calves fed a MR supplemented with a DFM, BYCW or combination of DFM and BYCW did not affect growth or performance compared with calves fed a non-medicated MR.

Key Words: calf performance, milk replacer, direct-fed microbial

M111 Effects of beta-glucans addition on milk replacer for Holstein dairy calves. M. E. Reis*[1], A. F. Toledo[1], A. P. Silva[1], A. M. Cezar[1], E. A. Fioruci[1], R. C. Silva[1], S. C. Dondé[1], L. Greco[2], and C. M. M. Bittar[1], [1]*Dept. Of Animal Sciences, College of Agriculture Luiz de Queiroz (ESALQ), University of Sao Paulo*, *Piracicaba, Sao Paulo, Brazil*, [2]*Kemin Animal Nutrition & Health Division South America*, *Valinhos, Sao Paulo, Brazil*.

The study aimed to investigate how addition of β-glucans can affect calf's growth performance, health, and fecal score. Holstein calves (n = 32) were individually housed in tropical shelters and blocked according to sex, date and weight at birth and randomly assigned to 1 of the treatments: (1) Control: milk replacer (14% solids, 24% CP, 18.5% Fat); (2) Aleta: milk replacer supplemented with β-glucans (Aleta, 2 g/d). All calves were bucket fed 6L/d of milk replacer and received

Table 1 (Abstr. M111). Performance of calves supplemented or not with beta-glucans

	Treatment			*P*-value[1]		
Item	Control	β-glucan	EPM	T	A	T×A
Concentrate intake, g DM/d	251.86	311.13	31.11	0.17	<0.01	0.40
ADG, kg	0.276	0.328	0.04	0.10	<0.01	0.06
FE	0.238	0.290	0.03	0.08	0.05	0.04
Birth BW	36.17	36.60	1.33	0.52	—	—
Final BW, 8 wk	51.53	56.35	2.17	0.05	—	—
Average BW, kg	42.76	44.86	1.58	0.13	<0.01	0.08
Fecal score	1.46	1.06	0.09	0.01	<0.01	0.10
Days with diarrhea	25.73	14.67	2.96	0.01	—	—

[1]T = treatment effect; A = age effect; T×A = treatment vs. age effect.

water and starter concentrate ad libitum starting on d 2. Feed intake, health score and fecal score were recorded daily. Data were analyzed as repeated measures using PROC Mixed of SAS.There was no difference on concentrate intake and ADG between treatments ($P > 0.05$). Feed efficiency was affected by treatment and age interaction ($P = 0.04$), with higher FE for β-glucans supplemented animals only in the third and fifth week of age. Supplemented calves presented lower fecal score ($P = 0.01$), decreased days with diarrhea ($P = 0.01$) and higher final BW ($P = 0.05$; Table 1). Overall, β-glucans supplementation may improve feed efficiency, final BW and fecal score during the preweaning phase.

Key Words: *Saccharomyces cerevisiae*, growth, diarrhea

M112 Effects of dietary organic acid and plant botanical supplementation on growth and hematological profile in Holstein calves transitioning from milk replacer to starter.

B. N. Tate*[1], A. B. P. Fontoura[1], V. Sáinz de la Maza-Escolà[1,2], J. T. Siegel Nieves[1], F. Wang[1,3], L. F. Wang[1,4], M. E. Van Amburgh[1], E. Grilli[2,5], and J. W. McFadden[1], [1]*Cornell University*, *Ithaca, NY*, [2]*University of Bologna*, *Bologna, Italy*, [3]*China Agricultural University*, *Beijing, China*, [4]*Henan Agricultural University*, *Zhengzhou, China*, [5]*VetAgro S.p.A*, *Reggio Emilia, Italy*.

Dietary organic acid and plant botanical (OA/PB) supplementation reduces gut membrane permeability and enhances growth performance in weaning piglets; however, the effects of OA/PB feeding in healthy young ruminants required investigation. Therefore, our objective was to investigate the effects of dietary OA/PB supplementation on early life growth performance in calves. In a completely randomized design, 36 bull and heifer calves were assigned to 1 of 3 groups (n = 12/group): control, low-dose OA/PB (75 mg/kg of BW); 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; AviPlus R; Vetagro, Italy), or high-dose OA/PB (150 mg/kg of BW; AviPlus R). Supplements were delivered as a twice daily bolus via the esophagus wk 1 through 8 of life. All calves received boluses equivalent for triglyceride. Calves were fed milk replacer (26% CP, 20% fat) at 1.7% of BW per day (dry matter basis) divided across twice daily feedings. Milk replacer intake was reduced by half at d 42 of age and terminated at d 49 of age. Starter (22% CP) and water were provided ad libitum for the duration of the trial. Body measurements were recorded weekly. Blood samples were collected before morning feeding for white blood cell and hematocrit analysis. The mixed model included the fixed effects of BW at birth, time, treatment, and their interactions with random effect of calf nested within treatment. Significance was declared at $P > 0.05$. Calves did not develop clinical disease and were deemed healthy. Hip height, BW, and average daily gain were not modified by treatment. Dry matter and metabolizable energy intake, and gain:feed were also similar across treatment groups. Dietary OA/PB supplementation did not alter total white blood cell counts; however, hematocrit scores were greater in calves fed high-dose OA/PB, relative to other groups ($P < 0.05$). We conclude that dietary OA/PB supplementation does not modify growth performance or alter white blood cell populations in healthy Holstein calves.

Key Words: dairy calf, organic acid, weaning

M113 Clinical evaluation of diarrhea calves submitted to therapeutic protocols containing sulfonamides with different routes of administration.

R. Klaus[1], L. V. Vieira[1], A. D. C. de Matos[1], U. S. Londero[1], J. Halfen[1], V. R. Rabassa[1], E. Schmitt*[1], R. A. Pereira[2], M. N. Corrêa[1], A. A. Barbosa[1], F. A. B. Del Pino[1], J. Feijó[1], and C. C. Brauner[1], [1]*Federal University of Pelotas*, *Pelotas, Rio Grande do Sul, Brazil*, [2]*Laboratory Ibasa*, *Porto Alegre, Rio Grande do Sul, Brazil*.

Neonatal diarrhea is 1 of the most important diseases in the dairy calve production system, mostly by affecting negatively the growth and performance of young calve, which may be associated with low treatment effectiveness. This study was designed to evaluate the clinical, hematological and growth performance of calves diagnosed with diarrhea against 3 treatments using sulfonamides. Fourteen Holstein calves were divided into 3 groups according to the administration route: oral (ORAL, n = 5); injectable (INJ, n = 4), and oral plus injectable (ORAL + INJ, n = 5). Clinical evaluations and fecal scores were performed on d 0, 3, 5 and 7 in relation to the day of diarrhea diagnosis. Fecal samples were collected on d 0 and 7, to identify the bacterial agent, performed by PCR, antibiogram and coproparasitological exams. Measurements of chest perimeter, withers height, and croup width were performed weekly until 30 d of age, while weight up to 60 d. The INJ group presented better ($P < 0{,}05$) growth performance and a trend ($P = 0{,}06$) to lower fibrinogen when compared with the others. *Escherichia coli* was identified in 100% (n = 17) of collected samples and the virulence genes, identified by PCR: hlyA, stx1, estIa, and eae, that are characteristic of enterohemorrhagic *E. coli* (EHEC) a producer of shiga toxin, enterotoxigenic (ETEC) and enteropathogenic (EPEC), respectively. Of all samples collected, 82.35% (n = 14/17) were sensitive to the sulfonamides and 17.65% (n = 3/17) resistant through in vitro sensitivity test. An association was observed between sensitive samples and the stx1 gene. In this sense, the results suggest a greater prevalence of genes related to hemolysin. All treatments seem to be efficient by controlling body temperature and decrease the fecal scores of the treated calves. However, the INJ treatment seems to have a less inflammatory response and improvements on growth performance, being the better treatment for diarrhea caused by *E. coli*.

Key Words: antimicrobial, *Escherichia coli*, growth performance

Ruminant Nutrition: General 1

M114 Is dietary selenite transformed into elemental selenium by rumen micro-organisms? Comparison of mineral and organic selenium forms in cows. M. A. Hachemi*[1], E. Pinloche[1], M. De Marco[1], S. Fredin[2], and M. Briens[1], [1]*Adisseo France SAS*, *Commentry, France*, [2]*Adisseo USA Inc*, *Alpharetta, GA.*

The lower efficacy of mineral selenium (Se) forms for status improvement compared with organic ones is largely described. However, limited results indicate it could be related to the formation of elemental Se (Se (0)) by rumen microorganisms (RMO). To test this hypothesis, we fed several forms of Se and measured the whole total Se (Tot-Se) as well as the speciation of Se (0) in rumen fluid and plasma of cows. Eleven ruminally cannulated nonlactating Prim Holstein cows were divided into 4 groups and fed for 23 d either a control diet (hay and protein-energy concentrate) (CON; n = 2), or the same control diet supplemented with 10 mg of Se top-dressed as sodium selenite (SS; n = 3), zinc-L-selenomethionine (Zn-SeMet; n = 3 or hydroxy-selenomethionine (Selisseo 2% Se; OH-SeMet; n = 3)). Rumen fluid (RF) was collected on d 22 and 23 and blood for plasma was collected from the tail vein on d 22, both 4 h after morning feeding. Plasma and freeze-dried RF were used for Tot-Se and Se (0) measurement by ICP-MS and HPLC-ICP-MS, respectively. Data were analyzed with a Kruskal-Wallis test. Results of plasma Tot-Se were CON: 32; SS: 94; Zn-SeMet: 127; OH-SeMet: 147 µg Se/kg of fresh material with significant differences within groups ($P < 0.05$) except between CON and SS groups ($P > 0.05$). The concentration of Tot-Se in RF was not different between the 2 sampling days. The concentration of Se in RF was positively correlated with the concentration of Se in plasma ($R^2 = 0.96$) and followed the same hierarchy. Se (0) was measured in RF but was not detected in any group except the SS group. The proportion of Tot-Se present as Se (0) in the SS treatment was 42%. We conclude that plasma Tot-Se is representative of rumen Se level and confirmed the higher bioavailability of organic Se forms, particularly OH-SeMet. Those results also confirm a transformation of SS into elemental Se, which could explain the lower bioavailability for SS.

Key Words: elemental selenium, selenomethionine, cows

M115 Effects of *Lactobacillus*, cellulase, and molasses on fermented sugarcane bagasse ruminal fermentation and in vitro digestibility. S. So*[1], A. Cherdthong[1], and A. P. Faciola[2], [1]*Khon Kaen University*, *Khon Kaen, Thailand*, [2]*University of Florida*, *Gainesville, FL.*

The objective of the study was to evaluate the effects of lactobacillus, cellulase, and molasses when added to a 30-d fermented sugarcane bagasse on ruminal gas kinetics, nutrient digestibility, ammonia nitrogen, protozoa population, and fermentation patterns using an in vitro gas production system. A 2 × 2 × 2 (+1) factorial arrangement in a complete randomized design was performed in this experiment. The (+1) treatment referred to fermented rice straw without additives (RS), as a negative control. The 3 factors of interest were: (A) levels of *Lactobacillus casei* TH14 at 0 and 0.05 g/kg of fresh matter, (B) levels of cellulase at 0 and 0.02 g/kg of fresh matter, and (C) levels of molasses at 0 and 0.05 g/100 mL of distilled water. Data were analyzed using the Proc GLM procedure of SAS, and the response effects comparison of each factor were evaluated using orthogonal contrast. Compared with RS and fermented sugarcane bagasse, gas kinetics and cumulative gas were increased by additives ($P < 0.05$). Additives enhanced in vitro DM, OM, NDF, and ADF digestibility compared with the control treatments ($P < 0.05$). Ruminal pH and ammonia nitrogen were not significantly affected compared with the control treatments ($P > 0.05$). The molar proportion of acetate decreased while propionate molar proportion increased by additives ($P < 0.05$). Estimated methane production was reduced by additives ($P < 0.05$). In conclusion, fermented sugarcane bagasse with *Lactobacillus* and additives had greater in vitro digestibility and improved ruminal fermentation patterns when compared with fermented rice straw and fermented sugarcane bagasse.

Key Words: additives, protozoa population, gas production

M116 Effect of nitrate and hydrogen addition on methane production in vitro. M. E. Rendon*, S. L. Ratiff, J. McDermott, J. Scott, R. Rha, and R. Kohn, *University of Maryland*, *College Park, MD.*

It is theorized that nitrate (NO_3^-) decreases production of enteric methane (CH_4) by using hydrogen (H_2), a substrate for methanogenesis. We hypothesized that adding gaseous hydrogen (H_2(g)) with NO_3^- would reverse NO_3^- inhibition of CH_4 in vitro. The effect of NO_3^- and H_2(g) on CH_4 and volatile fatty acid (VFA) production was analyzed in 2 runs of in vitro fermentation of corn grain and timothy hay. The treatments with corn were control (C), C + NO_3^- (CN) and C + NO_3^- + H_2(g) (CNH); and for timothy hay were control (T), T + NO_3^- (TN) and T + NO_3^- + H_2(g) (TNH). Nitrate was included in the flask at 3% of DM. The 2 runs differed in where the H_2(g) (60 mL) was placed. In run one, the H_2(g) was placed in a balloon attached to the top of flask; and in run 2, H_2(g) was incorporated directly into the headspace of the flask. The effect of additive on total CH_4 production and VFA concentration were evaluated with a mixed model using JMP Pro 14.1 (SAS Institute Inc.). In run one, CH_4 production (mL) in C was 4.94 but was lower ($P < 0.03$) in CN (2.81) and CNH (1.61). In the same way, CH_4 production (mL) in T was 4.04 but was lower ($P < 0.0001$) in CN (0.71) and CNH (0.76). In run 2, both NO_3^- and H_2(g) negatively affected ($P < 0.006$) the production of CH_4 for C, CN and CNH (5.67, 2.81, 1.48 mL). The CH_4 production differed ($P < 0.0005$) in timothy hay treatments with highest in T (2.96) and lowest in TN (0.49) and TNH between the 2 (1.54). In the first run, for both corn and timothy hay, VFA production (% of total VFA) was not affected ($P > 0.05$). In the second run, production of acetic (3.88, 3.37, and 2.59%) and propionic (1.59, 1.35, and 1.03%) acids in C, CN, and CNH, respectively, decreased ($P < 0.001$) with inclusion of NO_3^- and decreased more with H_2(g). With timothy hay, acetic acid production decreased ($P < 0.02$) only for TN compared with T and TNH (2.92, 2.36, 3.08% for T, TN, TNH, respectively). Effects of NO_3^- and H_2(g) on VFA production were inconsistent. Addition of NO_3^- decreased methane production in vitro, and the effect was sometimes reversed by adding H_2(g).

Key Words: nitrate, hydrogen, methane production

M117 Milk production and nitrogen efficiency in Holstein cows supplemented with a natural additive, ValKalor, in a low protein content diet. J. Ferguson*[1], L. Baker[1], J. Bender[1], J.-P. Ricaud[2], M. Aoun[2], and D. Pitta[1], [1]*University of Pennsylvania, School of Veterinary Medicine*, *Kennett Square, PA*, [2]*Idena Inc*, *Sautron, Pays de la Loire, France.*

Forty Holstein cows [mean milk production (SD) and DIM, 40.8 kg/d (8.3) and 96.7 d (25.3), respectively] were randomly assigned to 4 treatment pens (3 primiparous and 7 multiparous/pen). The experiment was a switchback design, 2 dietary treatments offered to 2 pens for two 3-wk periods, separated by a 2-wk washout period. The basal TMR consisted of corn and sorghum silages, ground corn, and a 36% protein mineral mix. The TMR included 13 ppm monensin. Treatment was 200 g (20 g/cow/d) of ValKalor (*Hibiscus sabdariffa,* blend of essential oils, Idena) in 4.5 kg of ground corn top dressed on the TMR. The control diet was 4.5 kg of ground corn top dressed on the TMR. Daily feed samples and weigh backs were collected by pen and composited by period for chemical analysis. Pen TMR and weigh back amounts and DMs were measured daily. Daily milk per cow was recorded at am and pm milkings and summed for volume over the experimental periods. Individual cow milk samples were collected in the third week (Tuesday p.m. and Wednesday a.m.) of each experimental period, composited and analyzed for milk fat, protein, urea nitrogen, and somatic cell content. Nutrient content (% DM) of the control and treat-

Table 1 (Abstr. M117). Production results

					$P <$		
Item	Control	SEM	ValKalor	SEM	Treat	Lact	Treat × Lact
Milk, kg/d	35.02	0.87	34.58	0.87	0.0037	0.0001	0.0591
ECM, kg/d	37.64	1.92	39.22	1.92	0.2062	0.0001	0.7024
Milk Pro, %	3.27	0.05	3.32	0.05	0.2549	0.1708	0.4811
Milk Fat, %	4.18	0.15	4.24	0.15	0.7517	0.7967	0.8190
Prot. yield, g/d	1,097.8	23.6	1,148.4	23.6	0.0869	0.0001	0.3962
Fat yield, g/d	1,402.4	48.2	1,465.4	48.2	0.3587	0.0004	0.9087
SCC, x1000	40.6	0.2	31.6	0.2	0.0447	0.9185	0.5359
MUN, mg/dL	11.4	0.3	11.3	0.2	0.7132	0.3314	0.5727
DMI, kg/cow	25.4	0.5	24.9	0.5	0.0445	NA	NA
N intake, g/d	598.9	25.7	561.9	60.4	0.0355	NA	NA
Milk N, g/d	194.7	1.7	195.0	6.1	0.5508	NA	NA
N efficiency	0.33	0.01	0.35	0.03	0.0240	NA	NA
ECM/DMI	1.54	0.05	1.58	0.08	0.2879	NA	NA

ment TMR, respectively was (%, SD): CP, 14.8 (0.7), 14.1 (1.3); NDF, 32.4 (2.0), 33.5 (3.0); starch, 27.8 (1.7), 27.0 (2.3); fat, 3.9 (0.3), 3.6 (0.4).

Key Words: production, milk protein, N efficiency

M118 Effectiveness of precision feeding in reducing N excretion in dairy cattle. M. Terré*[1,3], L. Morey[2], D. Sabrià[3], and A. Bach[4,1], [1]*Ruminant Department, IRTA*, *Caldes de Montbui, Spain*, [2]*GIRO, IRTA*, *Caldes de Montbui, Spain*, [3]*EVAM, IRTA*, *Monells, Spain*, [4]*ICREA*, *Barcelona, Spain*.

Eleven primiparous and 17 multiparous Holstein dairy cows (723 ± 3.1 kg of BW; 34.5 ± 1.69 kg/d of milk;159 ± 17.7 DIM) were blocked by parity and DIM, and randomly assigned to a conventional (CONV) feeding scheme based on a unique TMR or to a precision feeding scheme (PREC) for a 21-d period. The CONV group was offered a TMR (1.63 Mcal/kg DM, 16.5% CP), and PREC cows were fed a partial mixed ration (PMR; 1.59 Mcal/kg DM, 13.5% CP) and a concentrate feed supplement, which contained different proportions of soybean meal, corn, and wheat middlings according to animal estimated needs above the PMR consumption twice daily in the milking parlor. Daily needs were calculated using NRC equations (2001) with a rolling average of performance data (milk yield and quality, and BW) from 10 preceding days, and subtracting the nutrients consumed from the PMR computed also using a rolling average from 10 preceding days. Daily TMR and PMR intake, milk yield and quality were daily recorded, and a N balance using spot sampling during the last 3 d of the study was performed. Animals following both feeding systems had similar milk yield, milk fat and protein content, and total DMI. However, CP intake was greater ($P < 0.05$) in CONV than in PREC cows (4.26 vs 3.70 ± 0.170, respectively, kg/d), and improvements ($P < 0.05$) in feed efficiency (1.47 vs 1.39 ± 0.170, respectively) and efficiency of N utilization (0.32 vs 0.29 ± 0.005, respectively) were observed in PREC compared with CONV fed cows. Total daily N urine excretion (197 vs 142 ± 11.7, respectively, g/d) and milk urea concentration (130 vs 77 ± 14.5, respectively, mg/dL) were greater in CONV than in PREC fed cows. Feeding cows using a precision feeding system based on daily milk yield and composition can reduce environment N excretion without impairing performance.

Key Words: efficiency, environment, nutrition

M119 Direct effect of lipopolysaccharide and histamine on permeability barrier of rumen epithelium. S. T. Gao, L. Ma, A. L. T. Zhu La, W. H. Liu, and D. P. Bu*, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*.

Disruption of rumen epithelium (RE) barrier is very common during subacute ruminal acidosis (SARA) with lipopolysaccharide (LPS) and histamine (HIS) increased in the rumen. However, the individual roles of LPS and HIS in the process of RE barriers disruption are not clear. The objective of the present investigation was to evaluate the direct effect of LPS and HIS on barrier function of RE using the Ussing chamber system. Rumen tissues (n = 8) obtained from slaughtered feedlot steers were tested for changes in permeability to fluorescein 5(6)-isothiocyanate (FITC) with LPS (1 mg/mL) and HIS (20 mmol/mL). The FITC was added at 8 mL (final concentration: 0.2 mmol/mL) in the mucosal side of the Ussing chambers to detect changes in permeability of RE, and 8 samples were collected at 20, 40, 60, and 80 min from the serosal side (2 samples in each time, one for FITC detection, the other one for LPS and HIS detection). At the end of the experiment, the tissues mounted in the chambers were collected and separated into 2 parts, one for morphological analysis and the other one for detections of mRNA abundance related to tight junction. The transepithelial short-circuit current (Isc) and tissue conductance (Gt) were recorded continuously. The data were subjected to statistical analysis using MIXED PROC in SAS 9.4, with time, treatment, and the interaction between treatment and time as fixed effects and period as repeated effect. Compared with CON, HIS increased the Isc (88.2%, $P < 0.05$), Gt (29.7%, $P < 0.1$#), and the permeability to FITC (1.23-fold, $P < 0.05$) of RE. The apparent permeability of LPS was 2.8-fold higher than HIS ($P < 0.01$). The structures of the stratum granulosum, the stratum spinosum, and the stratum basale of histamine treated RE were severely damaged. The mRNA abundance of *OCLN* in RE was decreased by HIS (2.13-fold, $P < 0.05$). As such, the results of the present study suggested a direct role of HIS in the processing of the disruption of RE barrier function even without the cooperation of acidification.

Key Words: lipopolysaccharide, histamine, barrier function

M120 Effect of leaves of six forest plants on rumen microbiota and fermentation characteristics in vitro. A. Ayemele Gnetegha[1], L. Ma[1], T. Park[2], J. C. Xu[1,3], Z. T. Yu[2], and D. P. Bu*[1,4], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Department of Animal Sciences, The Ohio State University*, *Columbus, OH*, [3]*World*

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This study screened the leaves of 6 forest plants (*Brassica rapa chinensis*, *Kalimeris indica*, *Calotropis procera*, *Portulara oleracea*, *Flemingia macrophylla*, and *Adansonia digitata*) at 4 different doses (0, 0.7, 0.9 and 1.1 mg/mL) for their ability to inhibit rumen protozoa in vitro. Rumen fluid was collected from 3 different dairy cows and cultured anaerobically for 24 h incubation in the presence of ground leaves of each plant and then counted under a light microscope. The protozoal cells were examined using scanning electron microscopy for potential changes in cell surface morphology. Total bacteria and total archaea were quantified as copies of 16S rRNA genes per mL culture samples using qPCR. The VFA and ammonia concentration were determined using gas chromatography and colorimetry respectively. Data were analyzed in a completely randomized design using the PROC GLM procedure of SAS 9.4. Out of the 6 tested plants, only *Calotropis procera* inhibited the genus *Entodinium*, which is the main culprit of intraruminal microbial protein cycling, and decreased ammonia nitrogen production ($P < 0.05$), while not suppressing the cellulolytic genera *Orphryoscolex* or *Eudiplodinium* ($P > 0.05$). The leaves of *C. Procera* damaged the *Entodinium* extracellular structure in a dose-dependent manner. Total bacterial and archaeal populations were not decreased ($P > 0.05$). Except for butyrate which increased ($P < 0.05$), the molar proportion of VFAs and total VFAs production were not altered ($P > 0.05$). Chemical extraction and analysis of the *C. procera* leaves revealed phenolics (48.98 ± 1.89 mg/g of dry extract), flavonoids (8.93 ± 0.89 mg/g), and alkaloids (3.61 ± 0.75 mg/g) as the major secondary metabolites. Future research is warranted to identify the specific secondary metabolite(s) that are responsible for the specific inhibition of *Entodinium* and the underpinning inhibition mechanisms.

Key Words: anaerobic culture, nitrogen efficiency, *Calotropis procera*

M121 Prediction of dry matter intake using linear regression of sensor, blood metabolite, and performance variables in mid-lactation cows.

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Development of useful predictions of dry matter intake (DMI) for individual cows on farms could allow for the assessment of the impact of nutritional interventions on feed efficiency and facilitate the inclusion of thousands of additional cows in genomic reference populations for feed efficiency. The objectives of this study were to develop and evaluate DMI prediction models using cow performance, blood metabolite, and sensor data. Mid-lactation primi- and multiparous Holstein cows (n = 62/replicate) fitted with SMARTBOW eartags were housed in a freestall pen with Insentec feeders (2 replications; 45d). Sensor data collected via SMARTBOW included time spent lying (LT), ruminating (RT), and standing at the feedbunk (FT), as well as activity classified as high-active, active, and inactive. Other data collected included metabolic body weight (MBW), BCS, milk yield (MY), milk energy yield (MilkE), as well as plasma glucose, nonesterified fatty acids (NEFA), β-hydroxybutrate, and triglycerides (TG). All variables were scaled using the full data set and used as predictors of scaled DMI in multiple linear regression models. A model search was performed via the dredge function of MuMIN in R (v. 3.6.2). A 5-fold cross-validation was used to evaluate prospective models based on RMSE, r^2, and concordance correlation coefficient (CCC). Variables selected by all top performing models included BCS, MBW, and MilkE. The best performing model included BCS, MBW, MilkE, RT, active time, and NEFA (RMSE = 0.49; $r^2 = 0.85$; CCC = 0.87). Models excluding metabolites included BCS, MBW, MilkE, RT, and active time variables and achieved similar performance (RMSE = 0.49; $r^2 = 0.84$; CCC = 0.87). Performance was slightly poorer for models excluding metabolites and milk composition data; the selected model included BCS, MBW, MY, RT, and active time (RMSE = 0.52; $r^2 = 0.82$; CCC = 0.85). Models using only sensor and MilkE data included MilkE, FT, and LT (RMSE = 0.68; $r^2 = 0.57$; CCC = 0.71). In conclusion, DMI can be reasonably predicted using sensor and performance variables with or without the use of blood metabolites.

Key Words: predictive model, feed efficiency

M122 Fatty acid metabolism may vary in dairy cows with high and low residual feed intake.

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Mammary uptake and sequestration of triglycerides has been demonstrated to be greater in cows with lower residual feed intake (RFI). The objective of this study was to evaluate the differences in blood metabolites and markers of hepatic function between multiparous cows with high and low RFI as determined during 7-wk feed efficiency studies (n = 3). Calculated RFI was determined by regressing energy intake on milk energy output, median DIM, metabolic BW, and ΔBW (PROC MIXED, SAS 9.4). Cows were ranked by RFI and the top (HighRFI; feed inefficient) and bottom (LowRFI; feed efficient) 15% of phenotypic RFI (n = 18/group) were selected. Plasma and serum samples were taken concurrently from the tail vessel (TV) and the subcutaneous abdominal vein (MV). TV samples were analyzed for aspartate aminotransferase, alanine aminotransferase, and albumin. TV and MV samples were analyzed for blood urea nitrogen, lactate, carnitine, and acylcarnitines. Mammary arterial-venous difference (AVdiff) was calculated and variables were transformed as needed. The effect of RFI group was determined using PROC GLIMMIX with fixed effect of group and accounted for heterogeneity of variance. Data are presented as (mean [95%CI]). LowRFI cows had lower ($P = 0.01$) TV concentrations of C3-carnitine (0.86 µM [0.69,1.06] vs 1.31 µM [1.04,1.64]) and C4-carnitine (0.25 µM [0.22,0.28] vs 0.33 µM [0.29,0.37]), while TV C18:1-carnitine tended ($P = 0.08$) to be greater in LowRFI cows. Concentrations of C4-OH-carnitine were greater ($P = 0.01$) and C18:1-carnitine tended to be greater ($P = 0.10$) in the MV of LowRFI cows. Interestingly, AVdiff of C4-OH-carnitine was lower ($P = 0.01$) and C3-carnitine and C4-carnitine tended ($P < 0.10$) to be lower in LowRFI cows. There were no differences in concentration or AVdiff of other markers quantified. Although acylcarnitine exchange across mitochondrial membranes and between tissues is not fully understood, acylcarnitines are intermediates of fatty acid oxidation and may serve as markers of metabolism. Differences in circulating acylcarnitine concentrations suggest potential differences in fatty acid metabolism between high and low RFI cows.

Key Words: acylcarnitine, feed efficiency, arterial-venous difference

M123 Biotin, folic acid, and vitamin B_{12} supplements: Their effects on glucose tolerance in early lactation.

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This study aims to evaluate glucose and insulin metabolism of early lactating multiparous cows receiving biotin (B8), folic acid (B9), and vitamin B12 (B12) supplements. Cows (n = 32) were assigned to either 1 of the 4 treatments from −3 to 3 wk relative to the calving: 1) no vitamin (B8-B9B12-); 2) 20 mg/d of dietary B8 (B8+B9B12-); 3) 2.6 g/d of dietary B9 and weekly intramuscular injections of 10 mg of B12 (B8-B9B12+); 4) B8 with B9B12 (B8+B9B12+). An intravenous glucose tolerance test (IVGTT) was performed at 25 (SD: 2) days in milk after a fasting period of 12 h. Glucose (0.3 g of dextrose/kg of BW) was administered in 7.6 min; blood samples were taken −20, −10, 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, and 120 min post-infusion. Positive incremental method was used to calculate area under the curve (AUC). Data were analyzed with Proc MIXED (SAS) according to a factorial arrangement 2x2 and log-transformed when needed. Basal glucose, nonesterified fatty acid and glucagon plasma concentrations did not differ among treatments ($P > 0.16$) and averaged 2.96, SE: 0.15 mmol/L, 1,392, SE: 109 µmol/L, and 113, SE: 8 pg/mL, respectively. Supplementary B8 had no effect on basal insulin concentration in B9B12- cows but increased it when B9B12 was given, 1.07, 95% confidence interval (CI): 0.89–1.29 and 1.57, 95% CI: 1.30–1.91 mU/L for B8-B9B12+ and B8+B9B12+, respectively (B8xB9B12 interaction, $P = 0.05$). Glucose AUC (274, SE: 22 mmol·120 min/L), clearance rate (1.99, SD: 0.2%/min) and time to half-maximal concentration (35.1 SE: 1.0 min) did not differ among treatments ($P > 0.57$). B8 had no effect on glucose peak height in B9B12- cows but increased it in B9B12+ cows, 12.7, CI: 12.2–13.3 and 13.8, CI: 13.3–14.4 mmol/L for B8+B9B12- and B8+B9B12+, respectively (B8xB9B12 interaction, $P = 0.05$). Insulin AUC was greater for cows receiving B8 (1,950, SE: 110 mIU·120 min/L) than cows who did not (1,120, SE: 106 mIU·120 min/L; $P < 0.0001$). Peak insulin concentration was also greater for B8 cows ($P = 0.03$). The greater insulin release with the B8 supplement suggests that this supplement reduced insulin sensitivity.

Key Words: biotin, folates, cyanocobalamin

M124 Peroxisome proliferator-activated receptor alpha pathway in dairy cows in a TMR vs. a pasture-based system.

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To assess the effect of feeding strategy on the peroxisome proliferator activated receptor α (PPARA) pathway, multiparous Holstein cows (n = 24, 664 ± 65 kg BW, 3.0 ± 0.4 BCS, spring calving) were assigned in a randomized block design to a total mixed ration (TMR) fed ad libitum (70% forage: 30% concentrate) (G0) or grazing plus supplementation (G1) from 0 to 180 d postpartum (DPP). The G1 cows grazed *Festuca arundinacea* or *Medicago sativa* in 2 (18 h) or one session (10 h) depending on heat stress (30 or 20 kgDM/d) and were supplemented with 5.4 kgDM/d of a commercial concentrate or offered TMR (50% of G0 offer). From 180 to 250 DPP, all cows grazed *Festuca arundinacea* (10 h; 30 kgDM/d) and were offered TMR (50% of G0 offer). Liver biopsies were collected at 35, 110 and 250 DPP and mRNA abundance of *PPARA* and downstream genes: liver fatty acid binding protein (*FABP*), carnitine palmitoyltransferase 1A (*CPT1*), 3-hydroxy-3-methylglutaryl-CoA synthase 2 (*HMGCS2*) and acetyl-CoA carboxylase 1 (*ACC1*) were studied using real time PCR. Data were analyzed using a mixed model that included DPP, treatment and their interaction as fixed effects. Energy-corrected milk yield decreased ($P < 0.01$) by 12 kg/d at 250 DPP. Expression of *PPARA* mRNA decreased ($P < 0.05$) 30% at 110 DPP, but was not affected by feeding strategy. However, *FABP* mRNA was 1.5-fold greater for G0 than G1 cows. Alongside, *HMGCS2* mRNA tended to be 1.6-fold greater for G1 than G0 cows, no differences due to DPP were observed. Hepatic *FABP* mRNA decreased at 250 DPP (0.78 and 0.71 vs. 0.47 ± 0.19 for 35, 110 and 250 DPP, $P = 0.07$), while CPT1 mRNA was reduced at 110 DPP (0.77, 0.42 and 0.71 ± 0.30 for 35, 110 and 250 DPP, $P < 0.01$). No differences were observed for A*CC1* mRNA. Expression of *PPARA* mRNA correlated positively ($r \geq 0.4$, $P < 0.01$), with *CPT1*, *HMGCS2* and *ACC1* mRNA consistent with its regulatory role in the expression of these genes. We conclude that increased *PPARA*, *CPT1* and *FABP* expression may be attributed to increased lipid mobilization, alongside *FABP* overexpression in G0 could be linked to enhanced fatty acid transport while *HMGCS2* overexpression in G1 could represent incomplete oxidation of fatty acids.

M125 Effect of inoculant dose and time of ensiling on the fermentation and aerobic stability of snaplage.

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We evaluated the effect of a microbial inoculant on the fermentation and aerobic stability of snaplage. Snaplage was harvested (19-mm chop length) in 2 years (different hybrids and different farms) at 62% and 63% DM, respectively. For each year, snaplage was untreated (CTRL) or treated with a low level of SilageProB (SP) (SP-L; American Farm Products, Saline, MI; application rate: 200,000 cfu of /g of *L. buchneri* (LB)/ g of fresh weight and 50,000 cfu of *Pediococcus pentosaceus*, 50,000 cfu of *P. acidilactici* and isolated enzymes from *Aspergillus oryzae* and *Bacillus subtilis*) or a high level of SP (SP-H; differing from SP-L in that the application rate for LB was 400,000 cfu/g).Treatments were applied as individual replicates in quintuplicate and ensiled in 7.5L bucket silos (density of 240 kg of DM/m^3) at 21? for 30 and 90 d. Data were analyzed by ANOVA as a completely randomized design in a factorial arrangement of treatments (2 d of ensiling × 3 inoculation levels × 2 years) using JMP with significance declared at $P < 0.05$. Inoculation had no effect of numbers of lactic acid bacteria at 30 d but it was increased by both levels after 90 d. Inoculation did not affect the concentration of lactic acid or ethanol. There was a 3-way interaction on the concentration of acetic acid. Inoculation had no effect on the concentration of acetic acid at 30 d but at 90 d, SP-H increased acetic acid compared with CTRL, but only in 1 of the 2 yr. Inoculation numerically, but not statistically, decreased numbers of yeasts in silage (CTRL = 4.42, SP-L = 4.35, SP-H = 4.06 log cfu/g fresh weight). There was a day × inoculation effect on aerobic stability as there was no difference in stability of silages at 30 d (67, 70, and 74 h for CTRL, SP-L, and SP-H, respectively). However, at 90 d stability was higher for SP-H (102 h) than CTRL (69 h) but it was similar to SP-L (82 h). These data show that for the strain of LB used in SP, improvements in aerobic stability were not observed after 30 d and a high dose of LB was required to show efficacy at 90 d.

Key Words: snaplage, microbial inoculant, aerobic stability

M126 Evaluation of gluconeogenic substances in fresh cows.

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This study evaluated the effect of feeding glucose precursors to fresh dairy cows on body condition score (BCS), milk production and composition, metabolic and physiological status. Thirty-two dairy cows were randomly assigned to treatments in complete block design for 42 d post-calving. Treatments (300g/hd/d each) were: (1) Control (ground corn), (2) dry USP-grade glycerol powder, (3) a glycerol/glycol-based product, and (4) glycol added to daily diet. A mixed model (GLM) with repeated measurements was used to analyze the treatments effects on BCS, BW, milk composition and yield, and blood metabolites. Significant results were defined at $P < 0.05$ and tendency at $P < 0.1$ (SAS, 1998). The results showed that mean BW was not affected by treatments although group 2 mean BW was greater in wk 6 than in Control group (698.7 ± 14 vs. 659 ± 15 kg; $P < 0.05$). Body condition score and chest circumference did not differ between treatments even BCS was lower in Control group. Average milk yield was numerically higher in Group 2 than in other groups (42.2 vs. (1) 40.9, (3) 36.2 and (4) 40.2 kg/d). All supplements made milk protein yield increase compared with Control group ($P < 0.05$). It was numerically higher in group 2 (3.14% vs. (1) 2.99, (3) 3.01 and (4) 3.07). Data analysis showed plasma glucose concentration to be higher in Group 2 ($P < 0.05$) than in Control group and tended to be higher than those measured in the Groups 3 and 4 ($P < 0.1$). Blood urea was not affected by treatments. NEFA concentrations in Groups 2 and 4 were lower than those in Control group (0.340 and 0.384 vs. 0.578 mmol/L respectively; $P < 0.05$). Similar results were found for BHBA concentrations (0.441 and 0.440 vs. 0.75 mmol/L respectively; $P < 0.05$). Immune cell concentrations in blood were significantly lower in Group 2 (white blood cells, neutrophils, lymphocytes and monocytes) compared with Groups 3 and 4. The overall results suggest that feeding USP-grade glycerol supplement (300 g/hd/d) in fresh dairy cows may help to combine good metabolic status and performance. Further research is needed with more numerous animals including intake measurements.

Key Words: Holstein fresh cows, glucose precursor, milk performance

M127 The effect of different milk feeding levels on starter intake and subsequent performance and health of calves pre- and postweaning. D. Ziegler*[1], H. Chester-Jones[1], and B. Heinz[2], [1]*University of Minnesota*, *Waseca, MN*, [2]*University of Minnesota*, *Morris, MN.*

One-hundred eleven (2 to 5 d old) Holstein heifer calves (39.2 ± 0.61 kg) from 2 commercial dairies were randomly assigned to 1 of 3 milk replacer (MR) treatments to evaluate the effect of different milk feeding rates on calf starter intake, performance and health pre- and post weaning from d 1 to 84. The study was conducted between September 2019 and January, 2020. All calves were fed a medicated (Decoquinate at 45.4 g/ton) texturized calf starter (CS; 18% CP as fed). Water and CS were offered free choice from d 1 to 56. All MR were formulated with all-milk protein and fed at a solids content of 13.5%. Treatments were as follows: (1) 20% CP:20% Fat non medicated MR, fed at 10.75% of BW 2× daily from d 1 to 35 and 1× daily from d 36 to weaning at d 42, (CON); (2) 28% CP:18% Fat MR formulated with Bio-Mos fed at 17.0% of BW 2× daily from d 1 to 21 and then switched to MR as in CON at 10.75% of d 1 BW 2× daily from d 22 to 35 and 1× daily from d 36 to weaning at 42 d, (MOD); (3) 28% CP:18% Fat MR as in MOD, fed at 17% of BW 2× daily from d 1 to d 42, then 1× daily from d 43 to weaning at d 49 (HI). Data were analyzed using the PROC mixed procedure of SAS and repeated measures analyses applied where appropriate. Total MR and CS intake were different ($P < 0.05$) across treatments 20.8, 57.5 kg for CON 27.1, 48.2 kg for MOD and 38.5, 38.4 kg for HI, respectively. Total DMI was not different ($P > 0.05$) averaging 35.1 kg intake. There was a significant increase ($P < 0.05$) in ADG d 1 to 56 and d 1 to 84 for HI compared with CON and MOD, averaging 0.85, 0.85; 0.75, 0.79; and 0.77, 0.79 kg/d, respectively. Average fecal scores d 1 to 56 were greater ($P < 0.05$) for HI and MOD compared with CON, (1 to 4; where 1 = normal, 4 = watery), averaging 1.42 compared with 1.33, respectively. The earlier weaning of calves fed MOD compared with HI MR feeding program resulted in greater CS intake for MOD, however they had similar performance (d 1 to 56) to CON fed calves negating early performance advantages.

Key Words: calf performance, calf starter, milk replacer

M128 Maintenance energy requirements of two Holstein genotypes managed under pasture-based system. D. Talmón*[1], M. Garcia-Roche[1], A. Mendoza[2], and M. Carriquiry[1], [1]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República*, *Montevideo, Uruguay*, [2]*Instituto Nacional de Investigación Agropecuaria*, *Colonia, Uruguay.*

Milk production from grazing cows is often lower than that observed in different feeding systems due to their greater maintenance energy requirements (MEm). Twenty-eight multiparous fall calving Holstein dairy cows of New Zealand (NZH; 547 ± 67 kg BW and 3.27 ± 0.20 BCS at calving; n = 14) and North American (NAH; 589 ± 43 kg BW and 2.95 ± 0.23 BCS at calving; n = 14) origin were used to estimate MEm in each genotype. Cows grazed a *Dactylis glomerata* and *Medicago sativa* mixed pasture with 15.1 ± 5.8 kg DM/cow/day of herbage allowance, above 5 cm of ground level, and were supplemented individually to supply 33% of the estimated daily dry matter intake as concentrate. Heat production (HP) was measured, at 115 and 192 ± 19 DIM, using the heart rate-O_2 pulse technique and retained energy (RE) in milk and tissue were estimated according to NRC using milk yield and composition and changes in BW and BCS, respectively. Metabolizable energy intake (MEI) was calculated as HP + RE. Data were expressed as kJ/kgBW$^{0.75}$/d. Linear regressions of dependency of HP on MEI (HP = b × MEI + a) and fasting HP (FHP; regression intercept, a), heat increment (regression slope, b), energy efficiency (k; 1 − regression slope, 1 − b) and MEm (FHP/k) were calculated for each Holstein genotype. Considering the complete data set (n = 1), total RE, HP and MEI ranged from 664 to 966 kJ/kgBW$^{0.75}$/d, 885 to 1167kJ/kgBW$^{0.75}$/d, and 1549 to 2133 kJ/kgBW$^{0.75}$/d, respectively. Regressions of HP on MEI were significant ($P < 0.05$) for both genotypes (Table 1). The MEm was 17% greater for NAH than NZH cows which may partially explain the lack of difference in milk-solid yield observed frequently between genotypes when both are managed under a pasture-based system. Moreover, average MEm of both genotypes was 46% above the proposed by the NRC model (540 kJ/kgBW$^{0.75}$/d) probably associated with grazing activity and the high proportion of herbage in the diet.

Table 1 (Abstr. M128). Relationship of heat production (HP; kJ/kg of BW$^{0.75}$/d) to ME intake (MEI; kJ/kg of BW$^{0.75}$/d) and calculated maintenance energy (MEm)

Genetics	r^2	FHP (a)	Slope (b)	km ($1 - b$)	MEm (FHP/km)
North American	0.41	569	0.33	0.67	853
New Zealand	0.57	463	0.37	0.63	729

Key Words: indirect calorimetry, grazing

M129 Evaluating different doses of probiotics on rumen fermentation, nutrient digestibility, and methane production using batch fermentation assay. L. P. Marroquin*, K. G. Arriola, P. Tian, F. X. Amaro, C. A. Nino de Guzman, I. Fernandez, P. Schmidt, A. Oyebade, H. Sultana, S. Lee, E. J. C. Duvalsaint, M. M. C. N. Agarussi, and D. Vyas, *University of Florida*, *Gainesville, FL.*

The aim of this study was to evaluate the effect of different doses of probiotic on in vitro rumen fermentation, nutrient digestibility and methane emissions using TMR or corn silage as substrates. Two probiotics (A: mixture of *Lactobacillus animalis* and *Propionibacterium freudenreichii*; and B: mixture of *Lactobacillus animalis, Propionibacterium freudenreichii*, *Bacillus subtilis* and *B. licheniformis*) were applied at doses 0 (Control), 1.63 (Low), 3.25 (Medium), and 4.87 mg/mL (High) with substrate (TMR or corn silage; 0.5 g; 1 mm) in triplicates for each run for a total of 3 runs in experiment. Rumen fluid was collected and pooled from 3 ruminally-cannulated lactating dairy cows 2 h after morning feeding. Buffered rumen fluid (52 mL) along with substrate and probiotics were incubated for 24 h at 39°C. Gas production was measured at 0, 6, 9, 12 and 24 h and methane concentration was measured after 24 h of incubation. Data were analyzed using PROC GLIMMIX of SAS. Dose of each probiotic was used as fixed effect while run was used as random factor in the model. Linear and quadratic contrasts were tested, and significance was declared at $P < 0.05$, while tendency was declared at $0.05 < P \leq 0.10$. No effects were observed with Probiotic A on dry matter digestibility (DMD), gas production or methane concentration, compared with Control. However, NDF digestibility (NDFD) tended to increase in corn silage with Probiotic A, compared with Control (30.9 vs. 24.6%; $P = 0.06$) while no effects were observed with TMR. No effects were observed with Probiotic B on DMD, gas production or methane concentration, regardless of substrate used; however, NDFD tended to increase in TMR, compared with Control (56.5 vs. 55.3%; $P = 0.05$) while no effect was observed with corn silage. In conclusion, regardless of the dose used, probiotics tended to improve NDFD; however, responses were substrate specific.

Key Words: corn silage, in vitro fermentation, probiotic

M130 In vitro evaluation of two additives with different mode of action on rumen protein degradability. A. Alvarado[1], A. Britos[1], A. Pérez-Ruchel[1], F. Gadeyne[2], and C. Cajarville*[1], [1]*Departamento de Producción Animal (IPAV), Facultad de Veterinaria, UdelaR*, *San José, Uruguay*, [2]*Royal Agrifirm Group*, *Apeldoorn, the Netherlands.*

This work aimed to evaluate the effects of including 2 additives with differing modes of action to a dairy cow diet on ruminal fermentation and protein degradability. Comparison was made with a diet containing a processed

Table 1 (Abstr. M130). In vitro rumen fermentation characteristics and DM, CP, NCP disappearances (d) of the diets[1]

						P-value		
Item	C	M	Phy	ABC	SEM	T	D	T × D
pH	7.1	7.1	7.2	7.1	0.01	0.139	<0.001	0.791
Gas (mL/d)	678	701	693	675	48.4	0.686	0.04	0.849
N-NH$_3$ (mg/dL)	8.4^{a}	6.8^{b}	6.5^{b}	8.3^{a}	0.54	<0.001	<0.001	0.805
DMd (%)	45.0^{a}	42.5^{b}	42.9^{b}	44.4^{a}	1.15	0.004	0.364	0.777
CPd (%)	50.3^{a}	41.6^{d}	45.4^{c}	47.8^{b}	1.03	<0.001	0.017	0.473
NCPd (%)	40.2^{a}	35.9^{b}	37.1^{b}	36.7^{b}	0.66	<0.001	0.002	0.176

[1] C = control; M = Mervobest; Phy = 1% DM phytogenetic mixture added to C; ABC = 1% DM AromabioticCattle added to C; T = treatment; D = day.

protein source. In a Rusitec system (6 fermentation units) 4 diets were evaluated: C: control (30% corn silage, 30% grass silage, 11.5% soybean meal and 28.5% corn; 17% CP, 30.5% NDF), M: positive control (Mervobest replacing regular soybean meal), Phy (1% DM of a phytogenetic mixture added to C) and ABC (1% DM of Aromabiotic Cattle added to C). A balanced incomplete block design was used, and 4 runs were performed with 3 diets in duplicate incubated at a time. Each run lasted 14d (7d adaptation period plus 7d of sampling). Ten grams of DM was incubated in nylon bags. Inoculum was obtained from 3 rumen fistulated cows fed grass silage ad libitum, 1.5 kg DM/d of corn grain and 1.5 kg DM/d of soybean meal. McDougall artificial saliva was infused at 3%/h. Samples were taken from the fermentation unit at the same time as the bags were changed. Gas production, pH, N-NH$_3$ concentration and disappearance of DM, CP and non-protein compounds (NCP) were determined. Mervobest usage and Phy inclusion decreased N-NH$_3$ concentrations ($P < 0.001$) and DMd ($P = 0.004$). The CPd was highest for C, followed by ABC diet, Phy diet and M showed the lowest CPd ($P < 0.001$). The NCPd was similar between M, Phy and ABC diets, while C diet showed the highest value ($P < 0.001$). Results indicate that Phy inclusion at 1% decreased in vitro rumen protein degradation similar to Mervobest use and more effectively than ABC.

Key Words: Rusitec, nitrogen disappearance, rumen fermentation

M131 Effects of pH and temperature on amylase and glucosidase activity of exogenous enzymes.

F. X. Amaro*, H. Warman, K. G. Arriola, A. T. Adesogan, and D. Vyas, *University of Florida*, *Gainesville, FL*.

The objective was to evaluate the effects of different pH and temperature levels on amylase and glucosidase activity of 10 exogenous enzyme preparations (E1-E3; α-amylase, E4-E7; glucoamylase, E8-E9; mix of α-amylase, glucoamylase and fungal protease, E10; α-glucosidase). Two independent 10 × 4 factorial experiments were carried out to evaluate the activities of enzymes at different pH (4, 5, 6 and 7; Exp. 1) or temperature levels (20, 30, 40 and 50°C; Exp. 2). Potato starch (1% wt/vol) was used as substrate and glucose was used as standard. For amylase activity, enzymes (diluted in citrate-phosphate buffer) were incubated in triplicate with 1 mL of substrate in a temperature-controlled water bath for 5 min, reaction was terminated by adding 3, 5-dinitrosalicylic acid, sample absorbance was read at 540 nm. For glucosidase activity, enzymes were incubated under similar conditions for 45 min; however, reaction was terminated by adding glucose oxidase and absorbance was measured at 500 nm. Glucose release (µmol /mL enzyme/min) was calculated for both amylase and glucosidase activity. Data were log normalized and GLIMMIX procedure of SAS was used to test for main effects of enzyme, pH (Exp. 1) or temperature (Exp. 2) and their interaction. In Exp. 1, an interaction effect (enzyme × pH; $P < 0.05$) was observed for amylase activity with maximum activity for E3 observed at pH 6.0 while pH 4.0 induced lowest activity for E10. For glucosidase activity E1, E2 and E10 showed no activity at pH levels tested; however, E4 had greatest activity at pH 5.0. In Exp. 2, an interaction between enzymes and temperature levels was observed ($P < 0.01$). Enzyme E3 had maximum amylase activity at 50°C while E10 had lowest activity at 20°C. Similarly, for glucosidase activity, interaction was observed for enzyme and temperature levels as E1, E2 and E10 had no activity while E7 showed maximum activity at 50°C. In conclusion, the present study confirms pH and temperature effects on amylase and glucosidase activity of exogenous enzymes. Hence, it is recommended to determine optimum pH and temperature of exogenous enzymes before inclusion in livestock diets.

Key Words: amylase, glucosidase

M132 Effect of direct-fed microbial on commercial dairy drylot.

J. Lefler*[1], S. Minini[1], A. Morandi[2], and M. Embree[1], [1]*Ascus Biosciences*, *San Diego, CA*, [2]*Independent Nutritional Advisor*, *Argentina*.

The rumen microbiome plays a key role in the digestion of feed components, allowing the dairy cow access to nutrients and compounds that are crucial for milk production and health. As such, optimizing the productivity of the rumen microbiome is an overlooked strategy for increasing the performance of the animal. This study evaluated the efficacy of an orally administered, daily microbial feed supplement (MFS; Galaxis, Ascus Biosciences, San Diego, California) on a commercial dairy. One-hundred and 40 one, multiparous Holstein cows (DIM = 23 +/− 15) were split into 2 groups, 1 of which was fed an MFS containing 2 native rumen microbes (*Clostridium beijerinckii* and *Pichia kudriavzevii*) mixed daily with a TMR ration (30% Corn silage, 11% soy flour, 23% ground corn, 8% mineral premix, 2% wheat straw, 8% cottonseed, 6% ground soybean shell, 5% alfalfa hay, 7% alfalfa silage). The MFS was administered daily for 216 d on farm. The cows were milked 3 times a day and milk components were measured monthly. Mixed effects model analysis was conducted with cow ID specified as the subject and an imposed autoregressive covariance via the R package "nlme." Estimated marginal means were subsequently generated to compare the difference in response between treatment and control across weeks of trial via the R package "emmeans." The treatment by week interaction was significant ($P < 0.001$) for daily milk yield, which exhibited a 2.8 L (7.9%) increase in milk yield across the entire trial period. Milk components showed small changes in yield (kg) between treatment and control – most of which were non-significant except for milk fat ($P = 0.01$), which showed a roughly 2.3% decrease across the entire trial period. Energy-corrected milk (ECM) increased roughly 1.4% across the entire trial period ($P = 0.4$). These findings demonstrate the promise of using microbial based feed supplements in the improvement of herd production.

Key Words: microbial supplement, *Clostridium*, *Pichia*

M133 Supplementation with sunflower or soybean oil: Ruminal fluid fatty acid profile in a Rusitec system. G. Casarotto[1], A. Britos[1], S. Carro[2], I. Vieitez[3], C. Dauber[3], and C. Cajarville*[1], [1]*Departamento de Producción Animal (IPAV), Facultad de Veterinaria, UdelaR, San José, Uruguay*, [2]*Departamento de Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, UdelaR, Montevideo, Uruguay*, [3]*Departamento de Ciencia y Tecnología de Alimentos (CYTAL), Facultad de Química, UdelaR, Montevideo, Uruguay*.

The aim was to evaluate the effect of including 2 levels of sunflower or soybean oil to a forage-based diet on the rumen fluid fatty acid (FA) profile. In a Rusitec system 5 diets were evaluated: C (control, 65% alfalfa hay and 35% concentrate; 16% CP, 33% NDF, 3.4% EE), SF6 (C + sunflower oil added until reaching 6% EE of diet DM), SF7.5 (C + sunflower oil added until reaching 7.5% EE of diet DM), SB6 (C + soybean oil added until reaching 6% EE of diet DM) and SB7.5 (C + soybean oil added until reaching 7.5% EE of diet DM), incubating 8g of DM in Nylon bags. Inoculum was obtained from 2 rumen fistulated cows fed with the C diet and McDougall artificial saliva was infused at 3%/h. Three runs of 10 d (5-d adaptation period plus 5 d of sampling) were performed. Samples were taken from fermentation units at the same time as the bags were changed. Ruminal fluid FA profile was determined by GC. Data were analyzed according to a repeated measures model and means separated by LSMEANS. Sunflower and soybean oils inclusion resulted in a decrease of C16:0 ($P = 0.003$) and increased levels of C18:1 *t*11 (*trans*-vaccenic acid, TVA; $P < 0.001$). Diets SF7.5, SB6 and SB7.5 decreased the concentration of saturated FA with respect to control ($P = 0.041$; Table 1). Results indicated that adding sunflower or soybean oils up to 7.5% EE of total DM in the diet, did not increase C18:2 *c*9 *t*11 (CLA) levels, but increased its precursor TVA and may decrease saturation process.

Key Words: rumen fermentation, lipids, fatty acid

M134 Influence of substrates on efficacy of exogenous glucoamylase on in vitro dry matter digestibility, pH, and gas production. L. Mu[1], K. G. Arriola[1], G. Hao[1], H. Sultana[1], A. Oyebade[1], F. X. Amaro[1], K. Almeida[1], C. Heinzen[1], J. O. Gusmao[1], C. A. Nino de Guzman[1], I. Fernandez[1], W. Li[2], S. Yu[3], and D. Vyas*[1], [1]*University of Florida, Gainesville, FL*, [2]*DuPont Nutrition & Biosciences, Wilmington, DE*, [3]*DuPont Nutrition & Biosciences, Aarhus, Denmark*.

The objective was to evaluate the effect of exogenous glucoamylase supplementation on ruminal in vitro dry matter (IVDMD), pH, and gas production of different cereal grains used for livestock production. Five substrates (4 mm; 0.5 g per F57 bag) including sorghum (micronized and whole), barley (whole and steam-flaked), and corn (steam-flaked); were incubated with exogenous glucoamylase (from *Trichoderma reesei*; 0.25 mg/g substrate DM) and buffered rumen fluid for 7 h in 6 replicates per run in 3 independent runs per experiment. Gas production was measured at 0, 2, 4, and 7 h while DMD, and pH was measured after 7 h of incubation. Rumen fluid was collected from 3 rumen-cannulated lactating dairy cows fed corn silage-based diet formulated based on NRC (2001) guidelines. Data were analyzed using the GLIMMIX procedure of SAS. Treatments and sampling time (for gas production) were used as fixed effects while run was considered random factor. Exogenous glucoamylase had no effect on DMD of micronized sorghum ($P = 0.21$); however, DMD was increased by 10, 32, 19, and 59% with whole sorghum ($P = 0.03$), whole barley ($P = 0.04$), steam-flaked barley ($P = 0.05$), and steam-flaked corn ($P = 0.04$). Cumulative gas production was increased with glucoamylase when whole ($P < 0.01$) and steam-flaked barely ($P = 0.03$) while tendency was observed with steam-flaked corn ($P = 0.07$). No treatment effects were observed on pH values except with steam-flaked corn where pH was reduced with glucoamylase supplementation (6.65 vs 6.81; $P = 0.03$). The interaction between glucoamylase and sampling time resulted in greater gas production with amylase treated whole and steam-flaked barley after 4 and 7 h of incubation ($P = 0.03$). Similarly, the interaction effect tended to increase gas production for amylase treated steam-flaked corn after 4 and 7 h of incubation. In conclusion, IVDMD was increased with exogenous glucoamylase supplementation when whole sorghum, whole and steam-flaked barley, and steam-flaked corn was used as substrate.

Key Words: amylase, in vitro dry matter digestibility (IVDMD), gas production

M135 Effects of calcium carbonate supplementation rate on metabolic acid-base status and feed intake of cows with compensated metabolic acidosis. H. Fujan*[1], T. Brown[2], L. K. Mamedova[1], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS*, [2]*Landus Cooperative, Ames, IA*.

Controversy exists regarding the amount of calcium (Ca) to feed with anionic diets. Potential negative impacts of high Ca diets on dry matter intake (DMI) and diminishment of induced acidosis are 2 areas of interest. In this study, 21 pregnant, nonlactating cows (57.4 ± 13.9 d prepartum) completing at least 1 lactation were used in a replicated 3 × 3 Latin square design. All cows were fed a diet providing a DCAD of −6.0 mEq/100 g of DM. Treatments were a) no supplemental Ca carbonate (LOW; 0.6% DM Ca), b) a moderate level of Ca carbonate (MOD; 1.2% DM Ca), or c) a high level of Ca carbonate (HIGH; 1.8% DM Ca). Daily DMI and water intake were recorded. Urine and blood samples were collected 6 h

Table 1 (Abstr. M133). Rumen fluid FA profile (g/100 g of FA)

							P-value		
Fatty acid	C	SF6	SF7.5	SB6	SB7.5	SEM	T	D	T × D
C16:0	12.12^{a}	9.75^{b}	9.10^{b}	9.08^{b}	9.25^{b}	0.826	0.003	0.643	0.990
C18:0	3.75	3.51	2.87	2.58	3.14	0.666	0.261	0.908	0.669
C18:1 *t*11	0.51^{b}	0.75^{a}	0.75^{a}	0.73^{a}	0.75^{a}	0.040	<.001	0.545	0.877
C18:1 *c*9	40.69	43.05	44.42	44.97	44.55	1.794	0.067	0.773	0.713
C18:2 *c*9,*c*12	30.07	30.94	28.87	28.20	28.80	0.846	0.125	0.181	0.981
C18:3n-3	0.48	0.36	0.24	0.32	0.21	0.093	0.268	0.572	0.786
C18:2 *c*9,*t*11	0.41	0.12	0.17	0.33	0.19	0.107	0.357	0.475	0.730
SFA	20.21^{a}	18.85^{ab}	17.66^{b}	17.77^{b}	18.31^{b}	1.166	0.041	0.689	0.579
MUFA	40.74	43.13	44.48	45.01	44.61	1.824	0.067	0.775	0.718
PUFA	30.55	31.30	29.11	28.51	29.01	0.885	0.112	0.214	0.988

post-feeding. Urine samples were analyzed for pH and concentrations of creatinine, ionized Ca (iCa), and deoxypyridinoline (DPD). Blood samples were analyzed for metabolic indicators of acid-base status (pH, partial pressure of CO_2, oxygen saturation, total carbon dioxide, bicarbonate (HCO_3^-), and base excess) as well as iCa, Na, and K using a hand-held biochemical analyzer. Data were analyzed to assess fixed effects of treatment and period and the random effect of cow. There was no effect of treatment on DMI ($P = 0.21$) or water intake ($P = 0.28$). Urinary pH increased linearly with increasing Ca carbonate ($P = 0.009$; 6.41, 6.62 and 6.73 ± 0.12 for LOW, MOD and HIGH, respectively). Treatment did not alter urinary Ca excretion (4.75, 5.20 and 5.24 ± 0.63 g/d for LOW, MOD and HIGH, respectively; $P = 0.67$) or DPD ($P = 0.45$) and had no effect on measures of acid-base status or minerals in blood. DMI was greater in Period 3 vs. 1 ($P < 0.01$), associated with a decreased urinary pH in Period 3 vs. 1 ($P < 0.01$). Urinary pH was greater ($P = 0.03$) for cows on HIGH vs. LOW treatment with no difference in Ca excretion, suggesting dietary carbonate rather than Ca as the influencing factor. When feeding high levels of Ca carbonate to moderately acidified cows, urine pH may not accurately reflect systemic acid-base status. This could compel producers to feed more anionic supplement to maintain a urinary pH target.

Key Words: DCAD, bone, anionic

M138 Feed efficiency indexes in crossbred Holstein × Gyr heifers and its effects on energy and nitrogen partitioning, blood metabolic variables and gas exchanges. D. C. Silva[1], J. P. Sacramento*[4], L. G. R. Pereira[2], J. A. M. Lima[3], F. S. Machado[2], A. L. Ferreira[3], T. R. Tomich[2], S. G. Coelho[3], R. M. Mauricio[4], and M. M. Campos[2], [1]*State University of Southwestern Bahia, Itapetinga, Bahia, Brazil*, [2]*Brazilian Agricultural Research Corporation EMBRAPA Dairy Cattle, Juiz de Fora, MG, Brazil*, [3]*Federal University of Minas Gerais, Belo Horizonte, MG, Brazil*, [4]*Federal University of São João del Rei, São João del Rei, MG, Brazil.*

The effects of divergent phenotypic classification in Holstein × Gyr (F1) heifers for residual feed intake (RFI), residual weight gain (RG) and feed conversion efficiency (FCE) in relation to digestibility, energy and nitrogen partition, CH_4 emission, blood metabolic variables and heat production were evaluated in this study. Thirty-five heifers were ranked into 2 RFI, RG and FCE groups: high efficiency (HE) and low efficiency (LE). The animals were housed in a tie-stall facility and received TMR (75:25, corn silage: concentrate, 175 CP/kg, 4084 kcal/kg GE and 422 g/kg NDF in DM). Digestibility was evaluated during 5-d of total collection of feces and 2-d of urine. Gas exchanges (O_2 consumption, CO_2 and CH_4 production) were obtained in open-circuit respirometry chambers. A completely randomized design was used and the data were analyzed by ANOVA and correlation study. The comparison of means between the divergent groups was performed by Fisher's test. High efficiency animals for RFI consumed less O_2 ($P = 0.04$) and produced less CO_2 ($P = 0.05$). Heat production (HP) was lower for HE-RFI animals (171 vs 178 kcal/d/BW0.75; $P = 0.05$). Methane production was positively correlated with RFI, ($P = 0.03$). The HE-RG had higher O_2 consumption (2562 vs 2425 L/d; $P = 0.03$) and higher CO_2 production (2689 vs 2562 L/d, ($P = 0.04$) in relation to LE-RG. The HE-FCE, had greater NFC digestibility ($P = 0.01$), higher positive energy balance ($P = 0.04$), and excreted (12.3 g/d), less N in urine ($P = 0.02$). HE-RFI animals had lower β-hydroxybutyrate concentrations (0.80 vs 0.88 mmol/L; $P = 0.02$) and HE-FCE had higher concentrations glucose (5.24 vs 4.92 mmol/L; $P = 0.01$) in relation to LE-FCE. High efficiency RG and FCE groups emitted less CH_4 per kg of weight gain than LE animals ($P = 0.01$ and $P = 0.04$) respectively. The differences in performance, nutritional and metabolic parameters between the HE and LE groups varied according to the efficiency indexes adopted.

Key Words: digestibility, methane, residual feed intake

M139 Meta-analysis of the effects of linoleic fatty acid intake on lactating dairy cow performance. F. Díaz*, J. Sánchez-Duarte, and A. Garcia, *Dairy Research Center, dellait, Brookings, SD.*

Linoleic acid (C18:2) is an unsaturated fatty acid commonly found in dairy ration feed ingredients that may inhibit milk fat synthesis. The objective of this study was to evaluate the effects of dietary C18:2 intake on lactating dairy cow performance. Sixteen trials that added corn (0.7–2.8% of DM) or soybean oil (0.5–7.4% DM) to the diet in 14 published articles (2000–2019) were included. A mixed model meta-analysis was conducted using the random effect of study weighing by the inverse of the standard error of the means squared. Intakes of oleic (C18:1), linolenic (C18:3), rumen unsaturated fatty acids load (RUFAL), and all possible 2-way interactions and quadratic effects were included in the models. Multicollinearity was quantified with the variance inflation factor (VIF). The best-fit model was chosen based on the lowest Akaike information criterion (AIC) and root mean square error (RMSE). Residual vs. fitted values and Q-Q plots were used to identify the heteroscedasticity and normality of the final models, respectively. Marginal and conditional R2 explained the variance of the final models. No interactions, but high multicollinearity (VIF ≥3), were observed between dietary C18:2 intake and intakes of C18:1, C18:3, and RUFAL. Therefore, intakes of C18:1, C18:3, and RUFAL were removed from the models. Increasing dietary C18:2 intake from 143 to 760 g/d linearly decreased DM intake, 4% FCM, milk fat concentration, and milk fat yield (Table 1). Milk protein concentration and yield, however, were not affected by increasing dietary C18:2 intake. Results of this meta-analysis show that ingredients containing high concentrations of linoleic acid should be limited in lactating cow diets when the objective is to maximize milk production performance.

Table 1 (Abstr. M139).

Item	n	Coefficient	SE	*P*-value	RMSE	AIC
DMI, kg/d	51	−0.00303	0.00138	0.03	0.06	230.4
4% FCM, kg/d	51	−0.00974	0.00349	0.009	0.08	308.0
Milk fat, %	51	−0.00181	0.00031	0.0001	0.16	63.9
Milk fat yield (kg/d)	51	−0.00054	0.00015	0.001	0.11	3.7
Milk protein, %	45	−0.00020	0.00014	0.15	0.07	−29.2
Milk protein yield (kg/d)	47	−0.00006	0.00006	0.38	0.04	−63.54

Key Words: fatty acids, dairy cow, meta-analysis

Small Ruminant 1

M136 Effects of condensed tannins from sainfoin on the milk fatty acid profile of ewes. C. Baila, S. Lobón, M. Blanco, I. Casasús*, J. Bertolín, and M. Joy, *Ctr Invest y Tecnol Agroal Aragon (CITA), IA2 (CITA-Universidad de Zaragoza)*, *Zaragoza, Spain*.

There is an increased interest in using local fodder legumes to improve the self-sufficiency of feed for livestock. Sainfoin (*Onobrychis viciifolia*) is a typical high-quality crop of Mediterranean areas with a medium-high content of condensed tannins (CT), however their effects on milk composition are not well known. The aim of the study was to evaluate the effect of CT from fresh sainfoin on milk production, chemical composition, and fatty acids during 4 wk of lactation. Twenty pairs dam-lamb were individually fed fresh sainfoin ad libitum plus 200 g/d of barley. The distribution of pairs dam-lamb were done according to ewe's BW (60.7 ± 6.15 kg BW) and BCS (3.3 ± 0.57), lambing date (06/04/2019 ± 0.95) and lamb weight at birth (4.1 ± 0.64 kg BW). Half of the ewes were daily orally dosed 100 g of PEG 4000/200 mL water per ewe as a tannin-binding agent (SF+PEG) and the other half received only water (SF). Once a week, ewes were milked and samples of sainfoin and milk were obtained and analyzed for chemical composition and individual fatty acids (FAs). The total saturated FA (SFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), PUFA n-3 and PUFA n-6 were calculated. The sums and the major FA were analyzed using mixed models, with week of lactation as fixed and ewe as random effects using the SAS statistical software (SAS v.9.3). Milk yield was affected by the interaction between treatment and week of lactation ($P < 0.001$). The SF ewes peaked at wk 2 and SF+PEG ewes peaked at wk 4. The chemical composition of milk was only affected by the week of lactation ($P < 0.001$). The contents of FA in fresh sainfoin were similar through lactation, except for the PUFA, which had the greatest content on the first week ($P < 0.05$). The SF had greater C18:0 (13.6 ± 0.36 vs. 11.8 ± 0.37), C18:2 n-6 (2.10 ± 0.05 vs. 1.70 ± 0.05), C18:3 n-3 (1.75 ± 0.06 vs. 1.22 ± 0.06), C20:5 n-3(0.11 ± 0.005 vs. 0.09 ± 0.005) and PUFA (6.49 ± 0.104 vs. 5.94 ± 0.109) and lower C16:0 (22.4 ± 0.25 vs. 23.4 ± 0.26), conjugated linoleic acid (0.70 ± 0.028 vs. 0.88 ± 0.031) and n-6:n-3 ratio (1.11 ± 0.038 vs. 1.34 ± 0.04) than SF+PEG. The differences between treatments smoothed as the lactation advanced, mainly in CLA and n-6/n-3. In conclusion, the CT affected the milk FA profile especially in the first week of lactation and their effect decreased along lactation.

Key Words: polyethylene glycol (PEG), milk fatty acid evolution, suckling period

Animal Behavior and Well-Being 1

T1 Effects of social contact from birth on feeding behavior and health early in life and after introduction to an autofeeder. K. N. Gingerich*, B. A. Hoffman, E. E. Lindner, and E. K. Miller-Cushon, *University of Florida*, *Gainesville, FL.*

We investigated how social housing in a calf's first weeks of life affects milk feeding behavior, health, and subsequent adaptation to an autofeeder. Holstein heifer calves were randomly assigned to individual (n = 16 calves) or pair-housing (n = 8 pairs) at birth and received milk via a teat bucket (8 L/d; twice daily). Calves were mingled between treatments and group-housed (8 calves/pen) at 13 ± 2 d of age and fed via an autofeeder (12 L/d). At birth and upon introduction to the autofeeder, we recorded the number of sessions (based on twice daily feed delivery or autofeeder checks) where the calf did not feed voluntarily and required assistance. We measured daily milk intake and health (scouring events) from birth and daily milk feeding behavior for 1 wk following introduction to the autofeeder. Data were summarized by pair of calves (averaging across adjacent and consecutively enrolled individually-housed calves) and were analyzed in a general linear mixed model with housing treatment as a fixed effect, pen as a random effect for post-grouping outcomes, and day as a repeated measure for milk feeding behavior. Social housing did not affect the number of assists required to learn to use the teat bucket (1.9; SE = 0.2; $P = 0.26$) or milk intake in the first 2 wk of life (6.1 L/d; SE = 0.2; $P = 0.7$). All calves developed scours and age when first diagnosed with scours did not differ (6.2 d of age; SE = 0.6; $P = 0.4$) but pair-housed calves tended to scour for fewer days (4.2 vs. 5.8 d; SE = 0.6; $P = 0.06$). Upon introduction to the autofeeder, calves did not differ in the number of assists required to feed voluntarily (1.5; SE = 0.2; $P = 0.7$) but previously individually-housed calves occupied the feeder for longer (34.9 vs. 26.6 min/d; SE = 3.1; $P = 0.003$) without affecting milk intake (7.3 L/d; SE = 0.5; $P = 0.6$) or visit frequency (4.0 visits/d; SE = 0.4; $P = 0.4$). These results suggest that behavior and learning ability surrounding milk feeding are minimally affected by social housing, but pair-housing in the first weeks of life had a positive effect on health and efficiency of autofeeder use.

Key Words: dairy calf, social housing, feeding

T2 Effects of early social contact on dairy calf reactivity to novelty following introduction to group housing. E. E. Lindner*, K. N. Gingerich, J. M. Rivera, and E. K. Miller-Cushon, *University of Florida*, *Gainesville, FL.*

Social housing affects how calves interact socially and respond to novelty, yet even when group-housed, dairy calves may be initially housed individually after birth. We hypothesized that calves housed individually for 2 wk at birth would be more reactive to novelty after social grouping, compared with calves reared with social contact from birth. Calves were randomly assigned to individual (IH; n = 16 calves) or pair housing (PH; n = 8 pairs) at birth, then mingled between treatments and group-housed (8 calves/pen) at 13 ± 2 d of age. Calves were exposed to behavioral tests over 4 d, beginning at 4 wk of age, where the calf was placed in an arena with a start box marked at the entryway and a stimulus placed 2.5 m away, to assess response to: open field test (no stimulus), novel object (ball), unfamiliar calf (2 calves placed in pens), and unfamiliar human. Latency to exit the start box and contact the stimulus, and duration of contact directed toward calf or human, were recorded continuously from video. Data were summarized by pair of calves (either pair-housed or adjacent individual calves) and analyzed in a general linear mixed model with early life housing treatment as a fixed effect and group as a random effect. Calves did not differ in their latency to exit the start box during the open field test (11.9 s; SE = 3.3; $P = 0.28$). In the novel object test, PH calves tended to exit the box faster (31.9 vs. 91.1 s; PH vs. IH; SE = 4.7; $P = 0.09$) and contacted the object faster (206.0 vs. 370.3 s; SE = 50.0; $P = 0.036$). The latency to contact the unfamiliar calf did not differ between treatments, but PH calves spent more time in contact (32.9 vs. 22.6 s; SE = 6.0; $P = 0.04$). The latency to contact the unfamiliar human also did not differ, but IH calves tended to suck on the unfamiliar human for longer (2.0 vs. 6.0 s; SE = 1.4; $P = 0.06$). These results suggest that social isolation for the first weeks of life affects behavior after social grouping, increasing neophobia and altering social behavior, resembling effects associated with longer periods of individual housing in the pre-weaning period.

Key Words: dairy calf, social housing, neophobia

T3 Effect of rearing method of calves during milk drinking period on their response to an open-field and maze test after weaning. J. Broucek*, M. Uhrincat, P. Kisac, and A. Hanus, *National Agricultural and Food Centre*, *Luzianky, Slovakia.*

The objective was to determine the impact of the length of receiving milk from its own mother or nursing cow on the calf's behavior. Holstein calves (n = 99) were assigned to 1 of 3 treatments: suckling of mother (SM; n = 33; 16 heifers and 17 bulls), suckling of a nurse cow (SN; n = 34; 15 heifers and 19 bulls), or housed in hutches (H; n = 32; 15 heifers and 17 bulls). For the SM treatment, calves were in a pen with their mother (milked beginning on d 2) to d 21 of life, allowed to suckle for 10 min 3 × daily (8:00, 13:00, 18:00), and were then group housed and provided 6 kg/d milk by teat bucket (21 d suckling; 63 d manually fed). For the SN treatment, calves were with their mother for 3 d and then placed with a nurse cow (number of calves per cow determined according to milk yield; 6 kg milk daily; 84 d suckling). For the H treatment, calves remained with their dam for 24 h and were then housed in hutches from d 2 to 56 and provided 6 kg/d milk replacer by teat bucket (1 d suckling; 83 d manually fed). From the 2nd day until weaning (84 d) the calves were offered concentrate and alfalfa hay ad libitum. After weaning, all calves were kept separately by sex in age-balanced groups. The speed of traversing the maze was recorded (6 tasks during consecutive 3 d, 8x14 m closed maze facility) at 6 mo of age. Open-field (6 tests during 3 consecutive days, arena 10x10 m, 9 squares) was performed at the 10 mo. The data were analyzed using a General Linear Model ANOVA. The average time of traversing the maze facility for 6 tasks was no significantly differed among treatment groups. At the open-field test, the difference compared with the grid crossings was significant at the 1st minute of the 1st test (SM 9.2 ± 4.2; SN 9.1 ± 3.5; H 7.1 ± 2.9; $P < 0.05$). The average of the grid crossings in all tests was tended to be highest in SM and the lowest in H. There were no significant differences between sex and sire in behavior in the maze or in the open field tests. The results show that the method used to rear calves may have an impact on their later behavior.

Key Words: calf, rearing, behavior

T4 Pre- and post-weaning performance of calves housed individually or in pairs. D. Ziegler*[1], H. Chester-Jones[1], B. Ziegler[2], A. Manthey[2], and E. Dufour[2], [1]*University of Minnesota*, *Waseca, MN*, [2]*Hubbard Feeds*, *Mankato, MN.*

The objective of this study was to determine the effect of paired versus individual housing on growth and performance of calves pre- and post-weaning. One hundred (2 to 5 d old) Holstein heifer calves (39.8 ± 0.56 kg) from 2 commercial dairies were randomly assigned to 1 of 2 housing treatments, either individually housed or housed in pairs (d 1 to 56) followed by group housing in pens of 6 calves/pen by treatment (d 57 to 112). The study was conducted between June and October, 2019. All calves were fed a non-medicated 24% CP: 20% fat milk replacer (MR; all-milk protein) fed at 0.28 kg in 2 L of water 2x daily from d 1 to 7, 0.43 kg in 2.98 L of water 2× daily from d 8 to 35 and 0.43 kg in 2.98 L 1x daily from d 36 to weaning at d 49. All calves were offered a medicated (Decoquinate at 45.4 g/ton) texturized calf starter (CS; 18% CP as fed) and water free choice from d 1 to d 56. Milk replacer was fed using nipples via a milk bar. Housing treatments were as follows: 1) Housed in an individual pen 2.29 X 1.17 m with solid panel dividers (IH) and 2) Pair housed in an individual pen 2.29 × 2.34 m with solid panel dividers (PH). Calves were blocked to treatments by weight in a randomized complete block design. Calves housed in pairs were analyzed as an average of 2 calves. Data were analyzed using the PROC mixed procedure of SAS and repeated measures analyses applied where appropriate. There was an increase in ADG (d 1 to 56) for PH calves compared with IH calves ($P < 0.05$), averaging 0.74 and 0.67 kg/d, respectively. Total CS intake (d 1 to 56) was higher ($P < 0.05$) for PH calves compared with IH calves averaging 35.4 and 29.3 kg, respectively. Intake of MR (d 1 to 49) was higher for PH calves compared with IH ($P < 0.05$) averaging 31.9 and 31.6 kg, respectively. There was an increase in ADG (d 57 to 112) for IH calves compared with PH calves ($P < 0.05$), averaging 1.04 and 0.97 kg/d, respectively when group housed. Under conditions of this study PH calves had increased gain and starter intake compared with IH calves (d 1 to 56) although this performance advantage was not maintained d 57 to 112 with both treatments averaging 135.2 kg on d 112.

Key Words: calf performance, paired housing, calf starter

T5 Effects of space allowance on activity and cleanliness of group-housed dairy calves.

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With increasing social housing for dairy calves, there is a need to refine housing management practices to improve performance and welfare. Our objective was to measure the effect of pen space allowance on calf activity patterns and cleanliness. Holstein heifer calves (n = 30; 5 calves/pen) were group-housed at 14 d ± 2.8 d of age (mean ± SD). After a 7 d adaptation, each pen (n = 6) was exposed to 3 different space allowances (3.7, 4.6, and 5.6 m^2/calf) in a random order, according to a replicated Latin square design with three 7-d periods (period 1, d 22–28; period 2, d 29–35; and period 3, d 36–42). Calves were provided milk replacer (12 L/d) ad libitum via an automated milk feeder and gradually weaned over 10 d, beginning at 48 ± 3 d of age. Lying time was measured continuously using electronic leg-based accelerometers. Calf cleanliness was recorded twice a week based on a scoring chart (1 = calf is clean or only dirty near feet, to 4 = thighs and legs dirty). We calculated daily standing time, bout frequency, and bout duration, and cleanliness scores were averaged across experimental period. We also calculated the coefficient of variation for activity outcomes at the pen level to determine the effect of space allowance on within-pen variability. These data were analyzed in a mixed model with space allowance, period, and order of exposure as fixed effects, and day as a repeated measure for activity data (with pen as subject). Cleanliness score (2.2; SE = 0.1; $P = 0.82$) was not affected by space allowance. Standing time (389 min/day; SE = 16.4; $P = 0.31$) did not differ between treatments. However, with greater space allowance, calves had more frequent standing bouts (22.6 vs. 20.3 bouts/d; 5.6 vs. 3.7 m^2/calf; SE = 0.96; $P < 0.001$) of shorter duration ($P = 0.0027$). The coefficient of variation for standing behavior outcomes all increased with decreasing space allowance ($P < 0.04$). Overall, these results suggest that reducing space allowance affects patterns of activity at the pen level, and increasing variability within the pen suggests that individual calves may be differentially affected.

Key Words: dairy calf, space allowance, resting

T6 Association between feed efficiency, milk production, rumen fermentation, and feed sorting behavior of lactating dairy cows.

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Feed sorting behavior has implications for rumen health and milk production. The objective of this study was to examine the associations between feed efficiency and feed sorting, activity, milk components, and rumen pH and fermentation parameters. Sixteen lactating dairy cows were enrolled at 80 ± 15 DIM and data were collected during a 4 d period repeated over 3 consecutive weeks. Feed efficiency was defined as milk production per unit of DMI. Fresh TMR and orts samples were collected daily from individual cows for particle size separation. The 3 screens (19, 8, and 1.18 mm) and a bottom pan resulted in 4 fractions (long, medium, short, and fine). Sorting was calculated as the actual intake of each particle size fraction expressed as a percentage of the predicted intake of that fraction. The pH of rumen contents was measured immediately after fluid collection using stomach tube and rumen samples were analyzed for ammonia-N (NH_3-N) concentration. Association between feed efficiency and predictor variables was analyzed with multivariable mixed-effects linear regression models. The model included the random effect of cow and weeks were used as repeated measures. Unconditional associations between feed sorting, resting time, and milk composition were examined and independent variables associated with the outcome variable ($P < 0.25$) were considered for inclusion in the final model. Feed efficiency was associated with sorting for long ration particles (0.013 ± 0.007; $P = 0.10$), fat percent (−0.12 ± 0.04; $P < 0.01$); protein percent (0.70 ± 0.11; $P < 0.01$), standing time (0.0008 ± 0.0003; $P < 0.01$), and lying duration (0.004 ± 0.002; $P = 0.10$). A weak association of feed efficiency was also observed with rumen pH ($P = 0.21$) and NH_3-N concentration ($P = 0.19$). Milk fat ($P = 0.10$) and milk protein yield ($P < 0.01$) were associated with sorting against long forage particles. In conclusion, sorting for long forage particles is associated with feed efficiency; however, lying behavior is more associated with feed efficiency.

Key Words: activity, efficiency, feed sorting

T7 Association of feed intake with body condition loss during the dry period.

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Body condition loss during the dry period has been associated with a myriad of transition cow diseases. Further, over conditioned cows at dry-off are those that suffer the greatest BCS loss. We used data from 2 studies to assess the relationship between BCS loss during the dry period (DBCS), DMI, and BCS at dry-off. One hundred cows at the University of Guelph (Elora, ON, Canada) were enrolled in 2 studies to investigate the effects of different diets on feeding behavior during the dry period. In both studies BCS and BW were recorded at dry-off and biweekly up to calving. DBCS was calculated by subtracting BCS at dry-off from the BCS at calving. Feed intake data were collected using electronic feed bins. DMI was calculated as % of BW (DMI%BW). BCS at dry-off was categorized as over-conditioned (BCS ≥3.5) and normal (BCS <3.5); no cows had BCS <2.75. Parity and milk production in the previous lactation were considered. Data sets were split into far-off and close-up periods, wk 8 to 4 and 3 to 0 before calving, respectively. To evaluate the associations between DMI and BCS at dry-off in each period, mixed linear regression models were built using DMI%BW as the outcome variable. For each model the following covariates were included: parity, previous lactation milk yield, study, and diets that the cows were enrolled on in each study. Experimental day was included as random slope and cow as random intercept. We used mediation analysis to evaluate the potential causal direct effect of BCS at dry-off on DBCS and the potential indirect effect mediated by differences in DMI. Independent of diet, parity, and previous lactation milk production, BCS at dry off was associated with differences in DMI%BW; over-conditioned cows had lower daily DMI%BW during the far-off (normal: 2.1 ± 0.1 vs over-conditioned: 1.8 ± 0.1%; $P < 0.01$) and close-up (normal: 1.9 ± 0.1 vs over-conditioned: 1.5 ± 0.1%; $P < 0.01$) periods. DBCS was only partially mediated by DMI%BW (10%; lower CI = 2% - upper CI = 27%; $P = 0.02$), while BCS at dry-off still had a direct effect on DBCS

($P < 0.01$). Overall, these results suggest that mechanisms in addition to feed intake are associated with body condition loss during the dry period.

Key Words: fat mobilization, eating behavior, mediation analysis

T8 The effect of increased stall slope on lying behavior, rumination, and milk production of lactating dairy cows. A. M. Wilson*[1], T. C. Wright[2], J. P. Cant[1], and V. R. Osborne[1], [1]*University of Guelph, Guelph, ON, Canada*, [2]*Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada*.

The objective of this experiment was to determine the short-term effect of an increased stall slope on the lying behavior, rumination, and milk production of lactating dairy cows. This experiment was the first part of a project aimed at investigating the fundamental design aspects of freestalls to develop an improved cow platform with the use of a minimal indexing apparatus. Sixty multiparous Holstein cows (199.3 ± 45.6 DIM; 33.7 ± 5.7 kg/d) were divided randomly into 2 groups (n = 30) and exposed to 2 treatments in a crossover design with 4 7-d periods. Each group was housed in a pen with 30 freestalls and switched pens weekly. One pen had a stall slope of 3.5% (sloping from the front of the stall to the rear; parallel to stall length), which was the standard at the research facility. The stalls in an adjacent identical pen were modified to create a stall slope of 7%. All other aspects remained the same between both pens. Rumination and lying behavior were recorded continuously using rumination collars and leg-mounted pedometers, respectively. Milk yield was recorded twice per day. Data were analyzed with repeated measures ANOVA (PROC GLIMMIX) with cow as sampling unit within group and week as repeated measure. All means were adjusted with Tukey Kramer adjustment (SAS v.9.4). Cows on a 3.5% slope spent more time lying (13.0 vs. 12.8 ± 0.4 h/d; $P < 0.001$) and had fewer lying bouts (9.6 vs. 10.2 ± 0.6 bouts/day; $P < 0.001$) than cows on a 7% slope. There were no significant differences in milk yield (33.9 and 33.8 ± 0.4 kg; $P > 0.05$) or rumination (590.7 vs. 588.6 ± 10.0 min/day; $P > 0.05$) for 3.5% and 7% slopes, respectively. Results suggest that an increased stall slope may have an effect on lying behavior. This could be due in part to the relatively shorter distance between the stall surface and neck rail, since the neck rail height was not altered for the 7% slope. The increased slope did not affect milk yield or rumination.

Key Words: behavior, stall, slope

T9 Slick-haired Puerto Rican Holstein cows spend more time grazing under sunlight than their wild-type counterparts. H. L. Sánchez-Rodríguez*, I. Colón-Rodríguez, A. Rivera-Camacho, G. Vázquez-Arnal, N. Pérez-Rosario, N. Cruz-González, K. Domenech-Pérez, and G. Muñiz-Colón, *University of Puerto Rico, Mayaguez Campus*, *Mayaguez, Puerto Rico*.

Slick-haired (SLICK) cattle have been visually associated with a greater capacity to graze under direct solar exposure than their wild type (WT) counterparts. However, empirical data on this variable is scarce due to the complexity of visually evaluated behavioral research. Thus, the present study employed automatic data loggers to compare solar exposure periods between hair coat groups. Ten SLICK (1.70 ± 0.26 lactations, 95.80 ± 15.24 DIM, and 536.09 ± 19.71 kg of BW) and 10 WT (1.70 ± 0.26 lactations, 92.30 ± 15.29 DIM, and 492.90 ± 13.56 kg of BW) Puerto Rican Holstein cows received a collar with a light sensor (HOBO Pendant MX) tied on top. Sensors recorded light intensity values every 10 s for 2 consecutive days in November 2019, with thermal humidity index ranging from 66.98 to 85.21. Cows were maintained under the farm's management conditions, with natural shade provided at the fences of the grazing paddocks. A cutoff light intensity value of 4624.62 lx (determined in a previous validation trial) was used to differentiate between shade or solar exposure. Daily milk yield (averaged from the week before the study) was also recorded. Data (from 0600 to 1800h; averaged by min) were analyzed by the FREQ and GLIMMIX procedures (SAS). Similar parities ($P = 1.00$), DIM ($P = 0.87$), and BW ($P = 1.00$) were observed between groups. Milk yield was greater in the SLICK than in the WT cows (25.65 ± 2.26 and 21.38 ± 1.76 kg/d, respectively; $P < 0.01$). The SLICK cows spent a greater proportion of the grazing daily time exposed to solar radiation than the WT (52.67 vs. 47.33%, respectively; $P < 0.01$). Hair type and solar exposure degree (shade vs. sunlight) interacted ($P < 0.01$), with greater light intensity values in the SLICK than in the WT cows during solar exposure (24702 ± 106.65 vs. 20518 ± 98.43 lx, respectively), but no differences between groups during shade exposure (1856 ± 7.03 and 1793.2 ± 6.53 lx, respectively). Slick-haired cows spent more time grazing under the sun than their wild type-haired counterparts.

Key Words: slick-haired cows, light intensity, solar radiation

T10 Automatic shade and solar radiation exposure assessment in dairy cattle using light sensors. H. L. Sánchez-Rodríguez*, I. Colón-Rodríguez, A. Rivera-Camacho, N. Pérez-Rosario, K. Domenech-Pérez, and G. Muñiz-Colón, *University of Puerto Rico, Mayagüez Campus*, *Mayagüez, Puerto Rico*.

Heat stress limits feed intake in cattle, but tropically adapted animals spend more time grazing under direct solar radiation than their temperate counterparts. However, the direct study (i.e., through constant visual observations) of the daily time a cow spends under shade or exposed to solar radiation is time and labor consuming, which may limit its utility in heat stress related research. Thus, the present study aimed to evaluate an automatic light sensor (HOBO MX Temp/Light) as a possible means to determine this variable. For this, 20 Holstein cows (2.8 ± 0.37 lactations, 165.60 ± 27.75 d in milk, and 549.73 ± 21.47 kg of BW) at Lajas, PR, were fitted with a nylon collar containing a light sensor on top. Sensors were programed to record light intensity values every minute. Cows were rotated from artificial shade (0837–0906 h) to direct sunlight (0907–0932 h) and then back to artificial shade (0933–1005 h). The light intensity values recorded during the shade and sun periods were compared by Proc GLIMMIX in SAS. Because the sensors provide light intensity values, a feasible way to convert numeric to behavioral data (i.e., exposure to shade or solar radiation) was also evaluated. The maximum light intensity value observed during the shade periods (4624.62 lx) was used as the cutoff value to create shade or sun categories using the *if* statement in SAS. The sensitivity of the created categories was determined by Proc FREQ in SAS. Light intensity was smaller during the first ($P < 0.01$) and second ($P < 0.01$) shade periods than during sun exposure (2282.40 ± 647.64, 1907.27 ± 626.28, and 51026.00 ± 682.25 lx, respectively). No differences in light intensity were observed between shade periods ($P = 0.62$). Sensitivity values of 99.37 and 100% were observed for the created shade and sun exposure categories. The evaluated sensors were able to differentiate if the cow was under shade or exposed to direct solar radiation. The obtained numeric light intensity values were easily converted to behavioral data, which may allow for the automatic assessment of this variable in heat stress related studies.

Key Words: solar radiation, light intensity, tropical cattle

T11 Can housing system affect automated estrus detection in Holstein dairy heifers? R. A. Figueiredo[1], G. Mazon*[2], and J. H. C. Costa[2], [1]*Embrapa Genetic Resources and Biotechnology*, *Brasilia, DF, Brazil*, [2]*University of Kentucky*, *Lexington, KY*.

The objective of this study was to evaluate the effects of dairy cattle housing systems (HS) on automated estrus detection in Holstein dairy heifers. Thirty-six heifers (14.7 ± 2.2 mo old; 297.7 ± 65.2 kg) were evaluated from March to June 2019. Heifers were fitted with a behavior monitoring collar (MooMonitor+, Dairymaster, Ireland) and independently randomly assigned in 1 of 3 HS (bedded-pack, BP; freestall, FS; pasture, PA) in a 3 × 3 Latin Square. Each period lasted 29d before heifers were moved to a different HS. All heifers had 2 wk for habituation, then their estrus was synchronized using a 7d protocol. The protocol started (D0) with CIDR insertion plus GnRH (100 µg). Seven days later (D+7), CIDR was removed, $PGF_{2\alpha}$ (25mg) was administered, and heifers received an estrus detection patch. Visual observations were conducted (1h/twice a day) and ultrasonography was performed on protocol days −7, +2 and +7 to evaluate the presence and diameter of the largest follicles and CL. True estrus was defined if a heifer was visually observed in standing estrus, had more than 50% of the estrus detection patch activated, and ovulated. Estrus detection rate was 78% and the ovulation rate was 83%. Sensitivity (proportion of heifers in true estrus with an automatic estrus alert), specificity (proportion of heifers not in true estrus without an automatic alert), and accuracy (proportion of heifers that were correctly automatically identified in estrus or not) were calculated for each HS and differences between HS were assessed using a mixed model. Overall sensitivity, specificity, and accuracy of the behavior monitoring collar estrus alerts were 80.8, 79.2, and 91.7%, respectively. No effect of the period was found (*P* > 0.41). There were no differences in sensitivity between HS (BP = 96.7 ± 4.2, FS = 93.0 ± 4.2, PA = 95.8 ± 4.2; *P* = 0.77), or in specificity between HS (BP = 83.3 ± 19.9, FS = 88.9 ± 19.9, PA = 50.0 ± 19.9; *P* = 0.41). Lastly, no differences in accuracy were found between HS (BP = 94.4 ± 6.2, FS = 91.7 ± 6.2, PA = 88.9 ± 6.2; *P* = 0.83). In summary, HS did not affect the sensitivity, specificity, and accuracy of the behavior monitoring collar.

Key Words: technology, precision, validation

T12 Utilizing an ear-mounted accelerometer to estimate dry matter intake in transition dairy cows. G. Mazon*, M. R. Campler, and J. H. C. Costa, *University of Kentucky*, *Lexington, KY.*

The objective of the current study was to evaluate the use of precision dairy monitoring technologies to estimate individual DMI in transition dairy cows. Holstein dairy cows (n = 35; 1.9 ± 1.1 lact.; 715.8 ± 115.6 kg) were enrolled in the study from −28 ± 3 d until 21 d post-calving. Cows were fed using intake feeders (Insentec, Marknesse, the Netherlands) that recorded individual DMI and fitted with ear tags (CowManager, Sensoor, Harmelen, the Netherlands). Cows were randomly divided into 2 data sets: training (n = 12) and validation (n = 23). The training data set was used to develop an algorithm to predict DMI based on mixed linear prediction models during the dry and fresh period. Prediction algorithms for the dry period included lactation number, BW, ear temperature, time active and inactive, and rumination time. The algorithm for the fresh period included lactation week, ear temperature, feeding, rumination, active and high active time. Predicted DMI was compared with actual DMI using Pearson correlation, linear regression, and Bland-Altman plots. The model was considered precise if the correlation coefficient and coefficient of determination were high (>0.70), and mean bias (predicted – observed DMI) from the Bland-Altman plot included zero within the 95% interval of agreement. The model was considered accurate if the slope from the linear regressions did not differ significantly from 1. Pearson correlation coefficients were moderate during the fresh period and low during the dry period (r = 0.55 and r = 0.39, respectively). The coefficients of determination were negligible (fresh R^2 = 0.30; dry R^2 = 0.13). Bland-Altman plots were acceptable with the mean bias ± sd being −0.78 ± 4.16 and 0.60 ± 3.1 during the fresh and dry periods, respectively. The slope of the predictive model was 0.70 [95% CI; 0.41–0.99] during the fresh period and 0.32 [95% CI; 0.06–0.58]. In summary, DMI prediction models for the fresh and dry periods were not deemed precise or accurate. Future research should consider machine learning when developing DMI predicting models for transition dairy cows.

Key Words: automation, modeling, precision dairy

T13 Characterizing drinking behavior from reticular temperature with artificial neural networks. A. E. Pape* and C. S. Ballard, *William H. Miner Agricultural Research Institute*, *Chazy, NY.*

The use of reticular temperature (RT) to assess drinking behavior would be a useful tool to evaluate animal health and well-being. Our objective was to compare the performance of a temperature threshold and an artificial neural network (ANN) for identifying drinking bouts. Four commercial dairy farms were monitored from June through September, 2019. Thirty high producing (52 ± 8 kg/d) focal animals were selected on each farm and administered a RT bolus. Five days of data were selected randomly for each cow, yielding 150 cow-days for each farm that were manually labeled for drinking bouts. Two algorithms were experimented with in R 3.6.2. The temperature threshold algorithm consisted of applying a threshold at a specified quantile of RT for each cow-day, with any point falling below the threshold being classified as a drinking bout. Alternatively, 10 ANN each with a single hidden layer containing 20 units used RT observations over a 70-min time window as predictors, with the label (bout vs. non-bout) for the window being the label assigned to the time point at its center. Both algorithms were evaluated by 3 metrics: (1) area under the receiver operating characteristic curve (AUROC) using time point as the unit of observation; (2) mean absolute error (MAE) of predicted number of bouts using cow-day as the unit of observation; and (3) MAE of predicted mean interbout duration, also using cow-day as the unit of observation. Of 150 cow-days for each farm, approximately 60% were used for training and approximately 40% for validation. ANN outperformed the temperature threshold on all 3 metrics and for all 4 farms (Table 1). For mean interbout duration in particular, ANN provide enough sensitivity (MAE <4 min) to monitor even mild behavioral responses to changes in management and environmental conditions.

Table 1 (Abstr. T13).

Farm	Algorithm	AUC	MAE, n bouts	MAE, interbout duration (min)
A	Temperature	0.983	0.51	11.30
	ANN	>0.999	0.08	1.52
B	Temperature	0.986	0.47	9.69
	ANN	>0.999	0.12	3.28
C	Temperature	0.987	0.36	7.62
	ANN	0.998	0.14	2.10
D	Temperature	0.988	0.47	10.94
	ANN	>0.999	0.02	0.32

Key Words: drinking behavior, reticular temperature, neural networks

T14 Assessing animal welfare: Deriving individual welfare phenotypes from existing milk recording data. S. Franceschini*[1], J. Leblois[2], F. Lepot[2], C. Bertozzi[2], and N. Gengler[1], [1]*ULiège - Gembloux Agro-Bio Tech*, *Gembloux, Belgium*, [2]*Association Wallonne de l'Elevage*, *Ciney, Belgium.*

Animal welfare is an increasing concern in dairy production. Consumers want an ethical production while farmers want to ensure the health of the animals. Animal welfare measurements at the herd level such as the Welfare Quality (WQ) Protocol already exist but are time-consuming and costly. Moreover, assessing the overall well-being at the animal level becomes a challenge as herd measures for welfare cannot be directly translated to the animal level. Two projects, active in the Walloon Region of Belgium, HappyMoo (Interreg NWE) and ScorWelCow, are trying to define individual welfare scores (IWS) and their prediction from routinely measured milk recording data, including mid-infrared spectral data representing fine milk composition. Data from WQ Protocol and routine milk recording was collected during the same timeframe in 18 dairy farms with 1386 cows, the majority being genotyped. Two approaches to assess and to predict individual animal welfare were developed. The first approach consisted of 2 steps: translating the WQ principles into IWS and predicting these from milk recording data. The variation observed in the first step while regressing WQ animal measures on WQ principles was considered representative of the biological variation between cows. IWS prediction Partial Least Square regression for the 4 principles of the welfare quality scores have R^2 between 0.65 and 0.77. Moreover, results from this first approach showed a significant welfare assessor effect suggesting that welfare measurements were strongly human interpretation-dependent. This suggested the need for an alternative approach. The second approach directly used milk recording data such as spectral data to cluster cows in different groups, bypassing a priori definition of welfare by WQ. Those groups were compared with results from the first approach and showed possible discrimination for herds with enhanced WQ score (specificity = 1.00 but sensitivity = 0.10) thus suggesting further unsupervised analysis. Based on this research, novel individual welfare traits could be developed allowing future genomic selection for improved welfare.

Key Words: animal welfare, mid-infrared spectra, machine learning

T15 Estrus prediction model for dairy Gyr heifers. V. Vilela Andrade[1], P. Arrigucci Bernardes[2], R. Ribeiro Vicentini[3], A. Penido Oliveira[4], and L. El Faro Zadra*[1], [1]*Instituto de Zootecnia*, *Sertãozinho, SP, Brazil*, [2]*Universidade Federal de Santa Catarina*, *Florianópolis, SC, Brazil*, [3]*Universidade Federal de Juiz de Fora*, *Juiz de Fora, MG, Brazil*, [4]*Empresa de Pesquisa Agropecuária de Minas Gerais*, *Uberaba, MG, Brazil*.

In view of the particularities of the dairy Gyr breed in terms of estrous behavior and of the importance of efficient estrus detection, precision technologies can assist in the monitoring of estrus-related parameters. This study aimed to evaluate variations in core body temperature using continuous data of reticulorumen temperature (RRT) and animal activity (ACT) during estrus of dairy Gyr heifers and to use these measures in prediction models. The animals were submitted to an estrus synchronization protocol. The data were obtained with bolus instruments administered in the reticuloruminal cavity of 45 heifers, which recorded information at intervals of 10 min. The mean RRT and ACT were compared at different time intervals in relation to estrus (established by visual observation of mounting acceptance in the field). Mixed models were used for ANOVA of the 2 variables. Logistic regression, random forest and linear discriminant analysis were tested as prediction models using RRT, ACT, time of day and temperature humidity index (THI) as predictors, and the presence and absence of estrus as the variable to be predicted. The mean RRT during the period corresponding to estrus were higher ($P < 0.05$) than the mean temperatures on the day before (+0.22°C) and after estrus (+0.36°C) for all times studied. These results can be attributed to the hormonal changes that occur during this period. The mean ACT was higher ($P < 0.05$) for all times after removal of the progestogen implant compared with the times of the previous day, which can be explained by the behavioral changes characteristic of this phase. Among the prediction models, the random forest model provided the best mean performance values using RRT, ACT, time of day and THI as predictors. The ability to correctly predict the period of estrus (sensitivity) was 51.69% and the ability to predict the period of non-estrus (specificity) was 93.1%. The accuracy (ratio between the number of events correctly classified by the total number of events) was 0.87. Changes in RRT and ACT occur during estrus, which are important variables to be included in prediction models.

Key Words: sensors, body temperature, Zebu

Animal Health 2: Calves

T16 The effect of a fish oil based-product and canola oil on blood lactate, rectal temperature, health, daily gain, and starter intake on Holstein dairy calves. P. Melendez*[1], C. Roeschmann[2], A. Baudo[3], S. Tao[1], J. Bernard[1], P. Pinedo[4], and F. Farcey[5], [1]*University of Georgia, Tifton, GA*, [2]*University of Chile*, *Santiago, Chile*, [3]*Abraham Baldwin Agricultural College*, *Tifton, GA*, [4]*Colorado State University*, *Fort Collins, CO*, [5]*National University La Pampa*, *Gral. Pico, Argentina*.

Morbidity rate is still high in modern dairy calf operations. Nutritional strategies based on n-3 FA to positively modulate the immune system have barely been studied in dairy calves. The aim was to assess the effect of a based-fish oil product versus canola oil (control) on blood lactate concentration, rectal temperature (RT), nasal (NS) and fecal score (FS), BW and starter intake (SI) in Holstein calves. The study was conducted at the Dairy Research Center UGA, Tifton Campus. Between September and November 2019, 30 calves were randomly assigned by sex at birth to 2 groups. Calves were fed a milk replacer (26% CP, 20% fat, 12.5% solution), 2 L in a.m. and 2 L in p.m. during wk 1. Then, 3 L in a.m. and 3 L in p.m. between wk 2 and 7, and 3 L in a.m. during wk 8. Starter and water were offered from d 3 until weaning. A group received 30 mL of canola oil (CAN) and the other received 60 g of a fish oil-based product providing 30 g of fish oil (FO), daily, at the a.m. feeding until weaning. Products were added to the milk solution in the feeding bucket. At 7, 14, 21, 28, 35, 49 and 56 d of age a blood sample, RT, BW, and SI were taken. FS and NS were obtained daily. Lactate was measured in blood through a handheld device (Lactate Meter, Nova Biomedical, Waltham, MA). Lactate, RT BW, and SI were analyzed by ANOVA for repeated measures (PROC MIXED). FS and NS were analyzed by PROC NPAR1WAY, SAS. There was a tendency for lactate to be lower in the FO than the CAN at d 7 ($P = 0.09$) and 28 ($P = 0.06$). Neither BW nor RT, and SI were different between groups ($P > 0.05$). There was a tendency for the overall FS ($P = 0.1$) and NS ($P = 0.053$) to be higher in the CAN than the FO throughout the trial (Table 1). We concluded that FO improved slightly health parameters of dairy calves.

Key Words: calf, omega-3 FA, lactate

Table 1 (Abstr. T16).

	Lactate (mmol/L)		RT (°C)		BW (kg)		SI (g)	
Day	CAN	FO	CAN	FO	CAN	FO	CAN	FO
1	—	—	—	—	36.3	35.5	—	—
7	1.76	1.31	38.8	38.7	38.8	38.7	9.92	13.8
14	1.49	1.27	38.8	38.8	38.4	39.7	49.4	33.7
21	1.10	0.98	38.7	38.9	40.6	41.3	68.1	70.5
28	1.55	1.06	38.9	38.8	43.4	45.4	123.9	170.2
35	1.06	1.31	38.7	38.8	48.8	49.9	381.8	397.9
49	0.72	0.81	39.2	39.3	56.7	58.5	1,016.4	1,072.2
56	1.10	1.06	38.7	38.6	62.2	60.0	2,010.9	1,898.1

T17 Colostrum supplementation with omega-3 fatty acids does not alter calf outcome on a commercial farm. J. Opgenorth*, L. M. Sordillo, and M. J. VandeHaar, *Michigan State University*, *East Lansing, MI*.

Our objective was to supplement colostrum with omega-3 fatty acids (n-3 FA) to provide anti-inflammatory mediators that may improve the immune response of neonatal calves. We hypothesized a colostrum supplement containing 60 mL of a 1:1 ratio fish:flaxseed oil with or without 200 mg α-tocopherol might provide an advantageous start to early life by enhancing health and growth. Calves were blocked by birth order and randomly assigned to 1 of 3 treatments: no supplement added to colostrum (CON); 60 mL 1:1 fish:flaxseed oil blend (FF); 60 mL 1:1 fish:flaxseed oil blend with 200 mg α-tocopherol (FFE). In total, 180 heifer calves (n = 60 per treatment) were enrolled on a commercial farm. Health was scored 3 times per week until weaning. Weight, wither height, and heart girth were measured after birth, 3 wk, and 8 wk of age to assess pre-weaning growth. A subgroup of 54 calves (18 blocks or 18 calves per treatment) were sampled 2 d (±8 h) after birth to evaluate oxidant status, serum total protein, and inflammatory gene and cytokine protein expression in blood after an in vitro LPS challenge as indicators of health and immunity. At 9 wk, calves were transported 18 h to another farm, and medical records were kept as an indicator of disease incidence up to 13 wk of age. Calf mortality was 1.8 percent which is below industry average, and exceptional health was observed throughout the study. Health scores and growth were similar regardless of treatment. Serum total protein indicated successful passive transfer in all calves, and oxidant status index was not affected by treatments on d 2 of age. Concentrations of tumor necrosis factor α (TNF-α) increased with LPS stimulation, but this increase was not altered by treatment. Likewise, leukocyte gene expression of TNF-α, interleukins 8 and 10, and cyclooxygenase-2 increased upon LPS stimulation, but the fold change did not differ with treatment. In conclusion, 60 mL 1:1 ratio fish:flaxseed oil colostrum supplement did not enhance pre-weaning calf performance. Supplementing n-3 FA in a one-time meal may not provide the anti-inflammatory benefits observed with continuous feeding.

Key Words: oxidative stress, inflammation, neonate

T18 The occurrence of diseases and their relationship with passive immune transfer in Holstein dairy calves submitted to individual management in southern Brazil. L. V. Vieira*, M. A. A. Weiller, J. P. Noschang, J. Halfen, L. F. Bragança, D. A. Moreira, C. Pizoni, J. O. Feijó, A. A. Barbosa, V. R. Rabassa, E. Schmitt, C. C. Brauner, F. A. B. Del Pino, and M. N. Corrêa, *Universidade Federal de Pelotas*, *Pelotas, Rio Grande do Sul, Brazil*.

The failure of passive transfer of immunity (FPTI) in newborn dairy calves has been closely related to an increased incidence of calf diseases. Therefore, this study aimed to evaluate the impact of failures in the transfer of passive immunity in cases of diarrhea and respiratory diseases. The calves used in this study were descendants of commercial dairy farms located in the southern state of Brazil. Fifty-three Holstein dairy calves were monitored from birth to 60 d of age. Health checks were performed daily to get the incidence of diseases. Growth performance including BW, withers height, croup width, croup height, and chest circumference were recorded on d 7, 14, 21, 28, and 60 of the experiment. The FPTI was detected through the evaluation of total plasma proteins (TP) and its effect on the health and performance of the animals was performed based on 2 cohorts [satisfactory immunity (n = 36); and not satisfactory immunity (n = 17)] divided by the TP evaluation. The incidences between the cohorts were compared using the chi-squared test. The relative risk was calculated by the number of exposed sick animals incidence divided by the incidence of disease in unexposed animals, with a 95% confidence interval, using the Epi program. The growth performance was compared between cohorts using the ANOVA method. Seventeen animals had FPIT, while 36 had TP concentration higher or equal of 5.5g/dL in the first 24 and 48 h of life, which can be associated with adjusted immunity transfer. All animals with FPTI were diagnosed with diarrhea and 76.5% of them had respiratory diseases. However, 27 calves without FPTI were diagnosed with diarrhea and 17 with respiratory disorders, resulting in an incidence of 75% and 47.2%, respectively. The FPTI increased the relative risk for developing diarrhea by 33%, while for respiratory diseases the increased was of 62%. The growth performance was not affected by the FPTI. In view of the above, we conclude that FPTI can be a predisposing factor for diarrheal and respiratory diseases in neonatal dairy calves.

Key Words: diarrhea, respiratory diseases, neonates

T19 Antimicrobial use and decision making with respect to treatment of respiratory disease in Canadian dairy calves. T. Uyama*[1], D. Kelton[1], S. LeBlanc[1], D. Léger[2], S. Dufour[3], J. Roy[3], H. Barkema[4], E. de Jong[4], K. McCubbin[4], M. Fonseca[5], L. Heider[5], and D. Renaud[1], [1]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*, [2]*Centre for Food-borne, Environmental & Zoonotic Infectious Diseases, Public Health Agency of Canada*, *Guelph, ON, Canada*, [3]*Faculté de médecine vétérinaire, Université de Montréal*, *St-Hyacinthe, QC, Canada*, [4]*Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary*, *Calgary, AB, Canada*, [5]*Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island*, *Charlottetown, PEI, Canada*.

Antimicrobial resistance in livestock is a growing concern due to possible transmission to humans. Thus, it is important to understand antimicrobial use in farm animals. Dairy calves receive antimicrobials for the treatment of respiratory diseases, but it is unclear under what circumstances antimicrobials are used. The objective of this study is to investigate antimicrobial use and case-specific information used in treating respiratory diseases in Canadian dairy calves. A total of 105 dairy farmers (Ontario: 31; Alberta: 28; British Columbia: 26; Nova Scotia: 20) were selected purposively and completed a questionnaire in person about calf health. First, farmers were asked, “Do you use antimicrobials to treat respiratory diseases in calves”?. Second, only those who used antimicrobials to treat respiratory diseases were asked, “What case-specific information do you use to select a respiratory disease case for antimicrobial treatment”?. Respondents were instructed to select all that apply from a list of 4 symptoms (elevated breathing/respiratory rate, spontaneous/induced coughing, fever, presence of nasal/eye discharge) or otherwise specified. The average herd size was 162 milking cows (range 36–560). Among 105 farmers, 98% used antimicrobials to treat respiratory diseases. Among those who used antimicrobials for respiratory diseases, 80% used “elevated breathing/respiratory rate,” 67% used “spontaneous/induced coughing,” 61% used “fever,” 48% used “presence of nasal/eye discharge,” and 33% used other characteristics as indicators to treat respiratory diseases with antimicrobials. Among 34 farmers who specified other characteristics than symptoms given, 38% used “lethargy” and 35% used “lack of appetite” as an indicator for treatment. Among 91 farmers who were asked whether they had a written treatment protocol for respiratory diseases in calves, 35% reported that they have the protocol and 97% of them were discussed with veterinarians. Treatment decisions could be refined with inclusion of additional simple criteria to identify calves that require antimicrobials for their health or welfare.

Key Words: dairy calf, treatment protocol, case-specific information

T20 The systemic inflammatory response to intramuscular endotoxin challenge in dairy heifers. T. M. Sullivan*[1,3], A. Sharma[1,3], K. Lamers[1,3], A. Canovas[1,3], B. Mallard[2,3], and N. A. Karrow[1,3], [1]*Ontario Agricultural College, Department of Animal Biosciences*, *Guelph, ON, Canada*, [2]*Ontario Veterinary College, Department of Pathobiology*, *Guelph, ON, Canada*, [3]*University of Guelph*, *Guelph, ON, Canada*.

Dairy cattle routinely face a variety of stressors. Specifically, climate change has resulted in more frequent heat stress events that increase the incidence of bacterial infections by inducing conditions like leaky gut syndrome, whereby the integrity of the intestinal epithelium is compromised allowing for luminal bacteria and bacterial lipopolysaccharide (LPS) to infiltrate the host’s bloodstream resulting in systemic stimulation of the innate immune system. LPS is a well characterized and biologically relevant microbe-associated molecular pattern that makes up the outer membrane of gram-negative bacteria and is known to contribute to inflammatory disorders including mastitis, acidosis and septicemia. In the present study, Holstein calves (n = 72) were intramuscularly challenged with 0 (saline), 100, 200 or 400 ng/kg of LPS, and serum was collected hourly for 8 h to profile changes in circulatory stress biomarkers. All LPS treatment groups demonstrated distinct cortisol responses at 2 h post-LPS challenge ($P < 0.05$). Additionally, animals challenged with 200 ng/kg ($P < 0.01$) and 400 ng/kg ($P < 0.001$) of LPS demonstrated significant changes in serum cortisol concentrations for up to 4 h. A total of 8 cytokines, including TNFα, IL-10 and IL-6, were found to be dose-dependently induced between 2 and 4 h. The expression of immune-related miR-29b ($P < 0.1$), miR-31 ($P < 0.05$), miR-223 ($P < 0.05$), miR-1246 ($P < 0.001$) were also increased significantly over time. The dairy cattle stress response is a dynamic process, and the present study suggests that there is a peak window of time when cortisol, miRNA and cytokines are induced to regulate the inflammatory response. Variability in the inflammatory response warrants further investigation to better understand the contribution of genetics and associations between immune health and the stress response.

Key Words: sustainability, stress, resilience

T21 Using a rapid blood test to study the impact of heat stress on the immune status of first-time pregnant heifers and their calves. Q. T. Huo*[1], I. Toledo[2], B. Davidson[2], G. Dahl[2], and J. Laporta[2], [1]*University of Central Florida*, *Orlando, FL*, [2]*University of Florida*, *Gainesville, FL*.

This study was to evaluate the impact of late-gestation environmental heat stress on the immune status of first-time pregnant Holstein heifers during the peri-parturition period and their newborn calves using a rapid blood immunity test. The test uses gold nanoparticles that act as pseudo pathogens to probe the immune activity in blood samples. Upon mixing the nanoparticle with a blood serum, proteins from the humoral immune system including antibodies and complement proteins react with the nanoparticles, leading to an aggregate formation that is detected using dynamic light scattering. The test score is positively correlated to the humoral immune activity in the blood. Pregnant heifers were assigned randomly to 1 of 2 treatments: heat stress (shade, HT; n = 16) or cooling (shade, fans, and soakers, CL; n = 15) for the final 60 d of pregnancy (d −60). Weekly blood samples were collected from heifers (from d −60 to one week after calving) and bi-weekly from their calves (n = 13 per treatment) after colostrum feeding (d 0 14, 28, 42 and 56). Serum samples were analyzed in the laboratory. Student *t*-test was used to analyze the difference between sample groups. This study found that the humoral immunity of pregnant heifers decreased as they approach parturition, reaching the lowest level around the day of calving. Heifers under HT have lower immunity score compared with CL heifers on the day of calving ($P = 0.03$). Newborn calves show similar immunity test scores as dams on d 0, due to passive immunity transfer through colostrum feeding. No statistically significant difference was found from calves born from HT dams versus calves born from CL dams. Regardless of the treatment, there was a gradual decline of humoral immunity in neonatal calves from d 0, reaching the lowest level between d 14 and d 21. Afterward, the immunity test score of the calves increase gradually likely due to the development of calf's own immune system. In summary, this study finds that heat stress negatively impacts the immune status of late-pregnant heifers at the day of calving, but the impact on their newborn calves is not significant.

Key Words: immunity, pregnancy, heat stress

T22 Microbial composition of fecal transplant inoculum from dairy calf feces.

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GI disease is the most common illness in pre-weaned dairy calves and can lead to dysbiosis, an imbalance of the microbial composition of the gut. Therefore, restoring the microbial composition of the gut is paramount to overcoming the effects of dysbiosis. Fecal microbial transplant (FMT) therapy offers the potential for restoring the microbiota balance by transferring fecal matter from a healthy donor into a diseased recipient. In this study, consistently healthy calves of different breeds 5–24 d of age with fecal scores ≤ 2 out of 4 were used as fecal donors to create the FMT inoculum. A total of 358 frozen fecal samples from 73 calves were combined to prepare a single FMT slurry. All fecal samples tested negative for *Salmonella* and were processed under aerobic conditions. Samples (~130g each) were combined in a commercial blender with 650 mL of 0.85% saline. Fecal material was sequentially sieved (mesh size 35 and 60) and the slurry was centrifuged (15 min at 6,000 rpm). Supernatant was removed and the gelatinous organic material above the pellet was vacuumed. The slurry was re-suspended in a 1:1.25 saline-10% glycerol solution. Composite feces was allocated into size 00 capsules (Capsuline enteric coated) which were placed within size 0 Capsuline gelatin capsules and frozen at −80°C before administration. Three samples (1g) of the final FMT product were sequenced for the V3-V4 region of the 16S rRNA gene. The average microbial composition of the FMT product samples at the phylum level included 56.9% Actinobacteria, 38.1% Firmicutes, 3.4% Bacteroidetes, and 1.1% Proteobacteria. At the genus level, the product consisted of 47.3% *Bifidobacterium*, 11.1% *Blautia*, 9.7% *Lactobacillus*, 8.5% *Collinsella* and 2.1% *Faecalibacterium*. The predominant species were *Bifidobacterium longum* (21.5%), *Bifidobacterium pseudolongum* (15.3%), *Collinsella aerofaciens* (8.5%) and *Faecalibacterium prausnitzii* (2.2%). Alpha diversity was quantified by the total number of observed species. The FMT product had an average of 200 observed species with an abundance of microorganisms associated with gut health (*Bifidobacterium*, *Faecalibacterium)*.

Key Words: fecal transplant, dairy, calves

T23 Identification of internal control genes via RNA-seq analysis for data normalization in fecal RNA isolated from dairy calves.

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The use of fecal material to isolate RNA and perform gene expression analysis is a novel method that has been recently used in humans, rodents, and neonatal dairy calves. In the past, our group has used several internal control genes (ICGs) to normalize gene expression with the aim to reach a suitable stability across samples. However, the identification of the most suitable ICGs, when using fecal RNA for relative mRNA expression via RT-qPCR is remaining. This study aimed to identify robust ICGs for normalization of RT-qPCR through fecal RNA-seq data. Fecal samples were collected from 6 healthy Jersey calves (5 wk old) and frozen in liquid nitrogen until RNA isolation. Total RNA was extracted from 100mg of feces through bead-beater homogenization with trizol, followed by purification through silica membrane columns. The TruSeq stranded Ribo-zero rRNA reduced library was applied to the fecal RNA samples before sequencing. The libraries were sequenced at 2 × 50 base pair read length (NGS; Illumina, NovaSeq S4) at the University of Minnesota Genomics Center. Processed reads were aligned to the bovine genome using HISAT2. Mapped genes were used as counts per million (CPM) to evaluate their stability across samples. Genes with stable expression across samples and medium RNA abundance (3.5 to 6.5 logCPM) were used to identify potential ICGs. The pairwise comparison method using geNorm software with a combination of expression ratio stability (M; the lower the M value, the higher the stability of expression ratio) and optimal number of ICG calculated by the pairwise variation (V) between the normalization factor obtained using the selected genes were used to evaluate the ICGs. The genes with the most stable expression ratio (M <0.20) among those assessed were *SMS*, *VPS37A*, *ACTB*, and *GAPDH*. The V-value (ideally <0.15) for these selected genes were V = 0.04. The level of variation and stability of these genes were clearly below the thresholds previously reported for reliability. Thus, the geometric average of the ICGs *SMS*, *VPS37A*, *ACTB*, and *GAPDH* is an appropriate method for normalization of fecal RT-qPCR data.

Key Words: internal control genes, fecal RNA

T24 Microbiome and resistome characterization of colostrum from selectively treated dry cows.

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Few studies evaluate the impact of selective dry cow therapy on preservation of milk microbiome or the presence of antimicrobial resistance (AMR) genes at freshening. Objectives were to characterize the microbiome and resistome in colostrum of low somatic cell count (SCC) cows that were treated or not treated with antibiotics at dry-off. Cows eligible for dry-off on 1 NY dairy, with histories of SCC ≤ 200k were enrolled. Cows were randomly assigned to receive intramammary antibiotics and teat sealant (ABXTS) or sealant only (TS) at dry-off. Composite colostrum samples (within 4h of freshening) and quarter milk samples (1–7 DIM) were subjected to culture. DNA extraction was performed on culture negative samples (ABXTS = 43; TS = 33). After DNA from cows of the same treatment group and parity was pooled (26 pools; ABXTS = 12; TS = 14), amplification using V4 universal primers and shotgun sequencing were performed. The resistome was captured using a custom RNA bait library. Reads were aligned to resistance and taxonomic databases. Analysis was performed in R: diversity measures were compared; differences in composition were tested with analysis of similarities (ANOSIM). The most abundant phyla were Firmicutes (70%), Proteobacteria (24%), and Bacteroidetes (3%). Shannon and richness diversity means were not different between TS and ABXTS ($P = 0.6, 0.6$). Overall microbiome composition was not different at the phylum (ANOSIM R = 0.004, $P = 0.4$), class (ANOSIM R = 0.04, $P = 0.2$), or order (ANOSIM R = 0.005, $P = 0.4$) levels. AMR gene accessions identified 9 classes of AMR in 14 samples (TS = 9, ABXTS = 5). The majority of reads aligned to gene accessions that confer resistance to aminoglycoside (TS = ABXTS each 35% abundance), tetracycline (TS = 22%, ABXTS = 54%), and β-lactam classes (TS = 15%, ABXTS = 12%). Shannon and richness diversity means for AMR class were not different between TS and ABXTS ($P = 0.03, 0.06$). Resistome composition was not different between groups at the class (ANOSIM R = −0.20, $P = 1$) or mechanism levels (ANOSIM R = 0.01, $P = 0.5$). While no differences were found for the microbiome or resistome in this study, a larger sample size, deeper sequencing, and additional methodology may be needed.

T25 The exfoliated fecal transcriptome (exfoliome) and its resemblance to the intestinal gene expression in dairy calves.

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Tissue biopsies and postmortem analysis are limitations to evaluate molecular markers at tissue-level in the gastrointestinal (GI) tract. Inflammatory condition and barrier function of the GI through a non-invasive approach such as fecal RNA gene expression analysis has been reported in dairy calves. To further explore this approach, we performed a comparative transcriptome analysis via RNA-seq in GI epithelial cells in comparison to the exfoliated fecal transcriptome (exfoliome) in dairy calves. Postmortem jejunum and fecal samples (n = 6/tissue) were collected from healthy Jersey calves (5 wk old) and frozen in liquid nitrogen until total RNA isolation. The TruSeq stranded Ribo-zero rRNA reduced library was applied to the fecal RNA samples before RNA seq, targeting 2 × 50 PE reads, using the Illumina, NovaSeq (S4) platform at the University of Minnesota Genomics Center. The sequencing generated 41.6 ± 9.0 and 36.8 ± 5.6 M paired end reads in fecal and jejunum samples, respectively. Processed reads were aligned to the bovine genome using HISAT2. Differentially expressed gene (DEG) analysis was performed using a quasi-likelihood test in the EdgeR R-package applying a False Discovery Rate (FDR) < 0.05 correction. Although a greater paired end reads were generated with fecal samples than jejunum, a greater (35 ± 9.0 vs 7.1 ± 3.4) number of unmapped reads were obtained in fecal samples than jejunum. A linear discriminatory analysis was used to confirm the congruency between exfoliome and jejunum transcriptomes. Among those DEGs, the water channel protein *AQP8* was upregulated in feces compared with jejunum. Cell membrane transporter genes (i.e., *SLC27A5* and *SLC16A6*), and genes involved in the metabolism of proteins and cell proliferation (i.e., *CHST4*, *B4GALNT3*, and *FGFBP1*) were upregulated in the fecal RNA. Further analyses are needed, including the commonalities (overlap) between fecal and jejunum transcripts, and enrichment pathway analysis, to confirm that fecal RNA can reflect the intestinal transcriptome.

Key Words: fecal RNA, transcriptome, noninvasive method

T26 Colostrum management practices on New York dairy farms.

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The study objective was to describe colostrum management practices on New York (NY) dairy farms. A convenience sample of 18 NY Holstein dairies with average (range) milking herd size of 1,409 (540 to 4,150) cows were enrolled between October 2019 and February 2020. Data about colostrum management were collected using a questionnaire. Descriptive statistics were performed on colostrum harvest, management, and feeding. Colostrum was harvested into buckets in the parlor (n = 12, 66.7%), hospital parlor (n = 2, 11.1%), or maternity pen (n = 4, 22.2%). Out of 14 farms that harvested colostrum in the parlor or hospital parlor, 9 collected 3X/d and 5 collected 2×/d. Out of 4 farms that harvested colostrum in the maternity pen, 3 harvested within 2h of calving and 1 harvested 1×/d. Just over half (n = 10, 55.6%) of farms discarded colostrum for visual abnormality, oversupply, or not meeting farm-specific minimum Brix. Colostrum was pooled (n = 7, 38.9%), heat treated (n = 3, 16.7%), refrigerated (n = 12, 66.7%), filled into bags or containers (n = 14, 77.8%), frozen (n = 8, 44.4%), or fed directly to the dam's calf (n = 2, 11.1%). Two (11.1%) farms used pre-cooled milking equipment to rapidly cool colostrum during harvest. Colostrum Brix and calf serum total protein was read by a refractometer on 14 (77.7%) and 5 (27.8%) farms, respectively. Average (range) first feeding of colostrum was 3.73 (2.84 to 3.79) L and 3.50 (1.89 to 3.79) L for heifer and bull calves, respectively. Farms fed a second feeding of colostrum for heifer (n = 14, 77.7%) and bull calves (n = 7, 38.9%), with an average (range) volume of 2.00 (1.89 to 2.84) L. The first colostrum was fed via esophageal feeder (n = 12, 66.7%), combination of bottle or esophageal feeder (n = 4, 22.2%), or using a bottle (n = 2, 11.1%). This feeding occurred within 2h (n = 17, 94.4%) or within 12h of life (n = 1, 5.6%). Median colostrum Brix determined from all colostrum harvest on 12 farms over a 9-d period averaged (range) 24.6 (21.5 to 26.4) %. These results provide insight into typical colostrum management practices on mid- to large-size NY dairy herds.

Key Words: colostrum, New York, management

T27 Coccidiostats and antimicrobials in the prevention and treatment of neonatal diarrhea in calves: Systematic review.

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The goal of this systematic review (SR) was to evaluate the current literature on coccidiostats, and antimicrobial drugs used for the treatment or prevention of calf diarrhea. A review protocol was developed in accordance with PRISMA-P guidelines. The literature search was performed on October and November 2019 using 5 electronic databases (CAB Abstracts, PubMed, Science Direct, Scopus and Web of Science). Eligible studies were non- and randomized controlled trials in English. Descriptive statistics were performed using Microsoft Excel. A total 2,703 publications were retrieved; 26 manuscripts met the inclusion criteria. The studies were about diarrhea prevention (61.5%) or treatment (38.5%). Most studies were performed between 2000 and 2009 (31.0%); the earliest study included was published in 1960. Most trials were conducted in North America (46.2%). Studies reported financial support from private (48.1%) or public (22.2%) sources. Trial size ranged from 3 to 259 calves/treatment group; only one study included sample size calculations. Calves were of HO (53.8%) breed; enrolment age ranged from 0 to 40 d (7.2 ± 10.7 d). Most studies were performed with naturally infected calves (65.3%). All trials had a control group (negative: 96.2%; positive: 3.8%). A total of 18 different treatments from 29 studies were identified. One (69%) or 2 or more (31%) treatments were evaluated per study; coccidiostats (51.8%) and antimicrobials (48.1%). Routes of administration were PO (80.8%), IM (15.4%) or a combination of PO and IV (3.8%). Treatment efficacy on prevention was assessed based on fecal score (100%), clinical status (80%), and fecal shedding (60%), while cure was evaluated based on fecal score (87.5%), fecal shedding (81.3%), and weight gain (81.3%). Positive effects were reported based on a reduction of incidence (23.1%; prevention studies) or cure (60%; treatment studies) of diarrhea after clinical signs and fecal score evaluations. Based on our SR, standardized methods to evaluate clinical outcomes are needed. Also, future studies should include sample size calculations.

Key Words: calf diarrhea, systematic review, antimicrobial

T28 Predicting morbidity and mortality using automated milk feeders: A scoping review.

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Automated milk feeders (AMF) provide producers with a tool that can be used to more efficiently raise dairy calves. AMF are computerized systems that allow for the easier implementation of a higher plane of nutrition to dairy calves. AMF also have the ability to track individualized data, such as milk consumption, drinking speed, and the number of visits to the feeder, that could be used to predict disease. The objective of this scoping review was to characterize the body of literature investigating the use of AMF data to predict morbidity and mortality in dairy calves during the preweaning stage. This review will list the parameters that have been examined for associations with disease in calves and identify gaps in knowledge. Five databases and relevant conference proceedings were searched. Eligible studies focused on the use of behavioral parameters measured by AMF to predict morbidity or mortality in preweaned dairy calves. Two reviewers independently screened titles and abstracts from 6,679 initially identified records. Of those, 386 studies were included and then assessed at the full text level. Ninety-three studies fed calves using an AMF and provided some measure of morbidity and mortality. Of these, 15 examined AMF parameters for associations with morbidity or mortality. The studies were completed in North America (n = 7), Europe (n = 6) or New Zealand (n = 2). The studies varied in sample size ranging from 30 to 1,052 calves with an average of 225 calves. Seven of the studies used a mixture of Holstein or Jersey (or both) heifers and bulls, while the rest used exclusively bulls (n = 4) or heifers (n = 3). The most common parameters assessed for associations with health included drinking speed (L/min) (n = 8), rewarded and unrewarded visits (n = 9), and amount of milk consumed (L/day) (n = 14). Morbidity descriptions (time at risk and case definition) varied between studies. This scoping review revealed a small number of studies examining use of data from an AMF to predict disease in calves, with potential challenges with comparability of outcomes. Further research is needed to determine the efficacy of these parameters in commercial settings.

Key Words: dairy calf, computerized feeder, AMF

T29 Evaluation of a blend of botanicals, prebiotic, and organic selenium on *Cryptosporidium* naturally infected dairy calves.

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Cryptosporidium (*C. parvum*) infection can increase intestinal permeability and diarrhea in neonatal dairy calves. Consumer cultures interest in animal welfare and scrutiny of prophylactic drugs is growing; and thus, a natural program was evaluated on a commercial dairy farm (100 milking Holstein cows). Throughout consecutive births, female calves were pairwise allocated into 2 groups: no program (CTL, n = 15) vs. treated (VHP, n = 16) with a veterinary health product (New-Start - Probiotech International Inc.) at 10 g/calf/d mixed into milk meals for the first month of life. New Start is a proprietary blend consisting of 1.335 ppm Se from a selenized yeast, MOS, *Yucca schidigera*, *Vitis vinifera,* and essential oils. At d2 and 30 of age, heart girth method was used to estimate calf live weight (LW) with a measuring tape. At d6, 9, 12, 15 18 and 30 of age, diarrhea scores were performed and fecal samples collected. *C. parvum* excretion was analyzed using the optical density method from fecal samples. Haptoglobin concentration (HAP, n = 6 per group) was completed at d2 and 14 of age by an ELISA kit. Statistical analysis was performed in SAS using the MIXED procedure with repeated measures with day, treatment (trt) and the interaction as the fixed effects. There was a day × trt interaction ($P < 0.001$) on LW via heart girth which was due to day ($P < 0.001$) as trt were not different ($P > 0.28$) at d0 (CTL: 50.6; VHP: 53 ± 1.6kg) or 30 (CTL: 64.3; VHP: 65.1 ± 1.6kg). *C. parvum* excretion peaked at d9 in both groups; however, VHP (0.60 ± 0.05DO) excretion was less ($P < 0.001$) than CTL (0.87 ± 0.05DO). Although there was no effect ($P > 0.05$) of trt, day or the interaction on HAP, when applied to a commonly used reference interval range of 0 to 0.5g/L, CTL (d2: 0.54; d14: 0.48 ± 0.17g/L) HAP approached the upper limit, but VHP did not (d2: 0.23; d14: 0.25 ± 0.17g/L) regardless of day. Diarrhea scores were not impacted ($P > 0.05$) by day, trt or the interaction, nor did they support the HAP and *C. parvum* excretion results. Additional research with larger population is warranted to confirm the observations on *C. parvum* excretion.

Key Words: dairy calf, cryptosporidiosis prevention, natural alternatives

T156 Association among serum IgG concentrations in newborn dairy heifers and fertility, milk yield, and survival to first lactation.

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The objective was to analyze the associations between serum IgG concentrations in newborn Holstein heifer calves (mean ± SE = 3.7 ± 1.7 d old) and fertility, milk yield, and survival to first and second lactation. A retrospective study was performed with data from calves (n = 193) born in 4 farms in CO over a 12-mo period (June 2014 to June 2015). Calves were enrolled to evaluate pre-weaned heifer health and productivity as part of a national study where serum IgG concentrations, health events, and ADG were analyzed. Calves were categorized by serum IgG concentrations into 4 categories: A = ≥25.0 g/L (n = 79); B = 18.0–24.9 g/L (n = 57); C = 10.0–17.9 g/L (n = 36); and D = <10.0 g/L (n = 21). Data were analyzed using SAS. Season and year of birth were included as covariates in the analyses. Outcome variables included survival to 1st and 2nd calving, age at 1st calving, 305-d milk yield for lactation 1, and days to conception after 1st calving. Binary outcomes (survival) were analyzed by logistic regression, while ANOVA regression was used for continuous outcomes. Models included serum IgG category, season and year of birth as fixed effects and farm as random effect. Overall, 143 (74.1%) calves reached 1st calving (A = 81.0%; B = 70.1%; C = 69.4%; D = 66.6%; $P = 0.3$) and 105 (54.4%) started a second lactation (A = 64.5%; B = 47.3%; C = 44.4%; D = 52.3%; $P = 0.1$). No difference was observed in the odds of culling in the 1st lactation by IgG category ($P = 0.4$). A significant difference in age at 1st calving (mean ± SE) for A (715 ± 8 d) vs. C (760d ± 12 d), and D (695 ± 16) vs C (760d ± 12 d; $P = 0.005$) was established. Milk yield was not associated with serum IgG category ($P = 0.3$). Average 305-d milk production (±SE) in 1st lactation was A = 8,750 ± 706 kg; B = 7,626 ± 847 kg; C = 7,345 ± 1,050 kg; and D = 8,517 ± 1,469 kg. Days to conception after 1st calving (±SE) did not differ among IgG categories (A = 113 ± 13 d; B = 152 ± 19 d; C = 131 ± 23 d; and D = 169 ± 31 d; $P = 0.6$). In conclusion, status of passive immunity, indicated by serum IgG concentration early in life, affected age at first calving but did not impact fertility, 305-d milk yield, or survival to or through the first lactation.

Key Words: heifer, immunity, survival

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T30 Subpopulations of non-starter bacteria increase in the draining and matting conveyors during extended production shifts. B. Selover*, J. Johnson, and J. Waite-Cusic, *Oregon State University*, *Corvallis, OR*.

Cheese production environments are susceptible to microbial contamination in places where the temperature, pH, and available water and nutrients are sufficient to support growth. Long production runs can also contribute to non-starter bacteria growing in production equipment. Sources of contamination could originate from incoming raw milk or from biofilms within the draining and matting conveyor (DMC) that are reduced, but not eliminated by sanitation. The objective of this study was to identify areas in a commercial cheese production facility where bacterial populations increase during the production day. Combinations of milk, whey, curd, and/or food contact surface samples were collected on at the beginning, middle, end of the day, and after sanitation on 15 production days. Samples were enumerated for bacterial subpopulations using MacConkey, m-*Enterococcus*, *Acinetobacter* Chromogenic, and *Pseudomonas* Isolation agars. Representative isolates with unique morphologies were identified using 16S rRNA Sanger sequencing. Bacterial counts from swabs inside the DMC were usually below detection limit (10 cfu/mL) after sanitation; however, all subpopulations increased over the 18 h production day. Bacteria were detected at low levels (<2.4 Log cfu/mL) at mid-day. Inside the DMC (near the weir) at the end of the production day, coliform counts reached 5.3 Log cfu/mL and *Acinetobacter* increased to 6.2 Log cfu/mL. Throughout the DMC, *Pseudomonas* and *Enterococcus* were detected at levels as high as 3.1 Log cfu/mL and were detected after CIP (*Pseudomonas:* 1.4 Log cfu/mL; *Enterococcus:* 2.6 cfu/mL). *Klebsiella*, *Escherichia*, *Enterobacter*, *Pseudomonas*, *Acinetobacter*, *Enterococcus* and *Streptococcus* were all identified by 16S rRNA sequencing in samples collected from the DMC at the end of the day. Coliforms and *Enterococcus* increased 3.4 Log cfu/mL and 1.2 Log cfu/mL, respectively, inside the DMC in the final 8 h of the production day. This sharp increase demonstrates the critical need to consider the time between sanitation events for these subpopulations and consider their impact on finished product quality.

Key Words: cheese, microbiology, processing

T31 Influence of goat milk composition and level of α_{S1}-casein on the yield of fat-free fresh cheese model. F. Pinto[1], J. L. Riveros[2], and R. A. Ibáñez*[2,3], [1]*Pontificia Universidad Católica de Chile, Facultad de Agronomía e Ingeniería Forestal, Escuela de Graduados*, *Santiago, Chile*, [2]*Pontificia Universidad Católica de Chile, Facultad de Agronomía e Ingeniería Forestal, Departamento de Ciencias Animales*, *Santiago, Chile*, [3]*University of Wisconsin-Madison, Center for Dairy Research*, *Madison, WI*.

In goat milk, the level of expression of α_{S1}-casein (CN) is associated with animal genetics and relates to the content of total solids of milk and cheese yield. In Chile, goat production is mainly extensive, non-specialized, and with mainly Creole animals with high genetic variability in both production and levels of total solids in milk. In recent years, goat milk production has experienced a considerable development and technification, forcing the genetic selection of herds toward increased production and yields, as required by market needs. In this study, we analyzed the relationship between the composition and levels of α_{S1}-CN in goat milk with the yield of fat-free fresh cheese made on a laboratory scale. A goat dairy farm located in the dryland area of Central Chile (33°32′45″ S, 71°35′00″ W, 154 m altitude) and comprised by 87 goats, was used for this study. Individual milk samples were collected at 200 ± 7 d (mean ± SD) of lactation and analyzed for composition, somatic cell count, levels of individual caseins by reversed-phase high performance liquid chromatography and cheese yield (kg cheese/100 kg milk) obtained from a miniature cheesemaking laboratory model based on milk composition and using 40 g of skim milk. Variables were evaluated according herd phenotype: Alpine (n = 4), Chilean Creole (n = 7), Crossbreeds (n = 20) and Saanen (n = 56); and levels of α_{S1}-CN in milk: high (>15% α_{S1}-CN/total CN), medium (10–15% α_{S1}-CN/total CN), low (5–10% α_{S1}-CN/total CN) and very low (0–5% α_{S1}-CN/total CN). A positive correlation ($r = 0.768$; $P < 0.05$) was found between protein content of milk and cheese yield. However, a low correlation ($r = 0.243$; $P < 0.05$) was found between the level of α_{S1}-CN in milk with cheese yield. These results suggest that standardization of cheesemaking protocols based on milk composition may reduce differences in cheese yield due to genetic variability of individuals in a herd.

Key Words: α_{S1}-casein, goat milk, cheese yield

T32 Perception of cheese aromas: The case of 2-nonanone. C. C. Licon*[1,2], H. Razafindrazaka[3], D. Pierron[4], and M. Bensafi[2], [1]*Department of Food Science and Nutrition, California State University*, *Fresno, CA*, [2]*Lyon Neuroscience Research Center, CNRS UMR5292, INSERM U1028, Université Claude Bernard Lyon 1*, *Lyon, France*, [3]*Institute for Advanced Study in Toulouse, Université Toulouse 1 Capitole*, *Toulouse, France*, [4]*Paul Sabatier University-Toulouse III*, *Toulouse, France*.

Olfaction is one of the key aspects that drive food enjoyment and preference; cheese is not an exception, its quality is defined by its flavor and aroma compounds. It is known that all cheeses share almost the same aromatic compounds: alcohols, aldehydes, or ketones, among others, but only a few aromas are responsible for cheese odor perception. Thus, it is of interest to investigate the perception of some of the major compounds in cheese. A good example is 2-nonanone, a methyl-ketone produced in Gorgonzola, ripened Ragusano, Camembert, and Brie due to enzymatic activity of molds (*Penicillium camemberti, P. roqueforti* or *G. candidum).* From a sensory perspective, this molecule has been commonly associated with malty and fruity notes but also as cheesy and pungent, having a dual perceptual nature. The objective of this work was to study the perception of 2-nonanone by studying its aromatic threshold and description. Eighty-seven participants were tested in 2017, with ages between 19 and 25 years old (mean 21 ± 1.4), 23 males and 64 females. The threshold of 2-nonanone was calculated using the best estimate threshold (BET) method using 5 ascending concentrations (0.1 ppb, 1 ppb, 0.01 ppm, 0.1 ppm, 1 ppm) with an alternative forced-choice task (using 4 flasks, 3 of them blank). We also asked the participants to rate the aroma (pleasantness, intensity, familiarity, edibility, irritation in a scale from 1 to 7 and aromatic description) at 0.1 ppm if they were able to detect it. The 2-nonanone was diluted in deodorized mineral oil and presented in a 15-mL flask (diameter 1.7 cm; height, 5.8 cm; filled with 5 mL of liquid). Results showed that the group BET for 2-nonanone in mineral oil was 14 ppb. We found that 13 participants (14.94%) were not able to identify any of the concentrations presented while 4 participants (4.6%) were able to identify all concentrations correctly. No significant correlations were found ($P > 0.05$) between the threshold and the perceptual attributes. The aroma was described as chemical, cheesy, fruity, floral and unpleasant. Further analysis is needed to determine if there is a correlation between the perception of this compound and cheese consumption.

Key Words: cheese aroma, perception, 2-nonanone

T33 Profiles of fatty acid composition in relation to water activity of powder goat milk stored under different storage time and temperature. R. Paswan*[1], A. Siddique[2], A. Mishra[3], and Y. W. Park[1], [1]*Fort Valley State University*, *Fort Valley, GA*, [2]*Auburn University*, *Auburn, AL*, [3]*University of Georgia*, *Athens, GA*.

Water activity (a_w) is important index for food quality, safety and storage stability, where a_w is closely related to bacterial growth. It was reported that

a_w affected the fatty acid composition of dehydrated bovine milk, because lipid oxidation in low moisture foods caused changes in fatty acid composition of the products. However, such reports on powdered goat milk (PGM) have been scarce. The purpose of this study was to determine fatty acid (FA) profiles in commercial PGM products in relation to a_w. Three batches of commercial PGM products were purchased from a local retail outlet in Warner Robins, GA, and assigned to 2 storage temperatures (ST) (4°C and 25°C) for 10 storage periods (SP) (0, 3, 7, 14, 21, 30, 60, 90, 120 and 180 d). Basic nutrient contents of the PGM samples were determined using the AOAC (1995) procedures. Water activity of the PGM samples was determined using the AquaLab water activity meter (cx-2; Decagon Devices). FA profiles of all experimental PGM samples were quantified using a gas chromatograph (GC-2010; Shimadzu), equipped with a fused silica capillary column (Supelco-2560), flame ionization detector and AOC-20s auto sampler. Results showed that oleic acid (C18:1) had highest, caprylic acid (C8:0) was the second highest, and behenic acid (C22:0) had lowest level among all the FAs identified in experimental PGM samples. The samples stored at 4°C had slightly higher FA values than those stored at 25°C, except for C18:0 and C18:1 at 4-mo storage. Majority of FAs tested were significantly affected by SP, while effects of ST were not significant for C8:0, C14:0, C16:0, C18:1, C18:3, C:20, C:22, C24:0 acids. No significant correlations (r) were found between a_w and fatty acids contents in the PGM stored at 25°C, whereas those r values of the samples stored at 4°C were significant for C8:0, C16:1, C18:1 and C18:2 acids. The C:8 content revealed the highest r value with a_w among all FAs analyzed. It was concluded that majority of FAs concentrations in the commercial PGM were affected by main factors, especially batch and storage period treatments.

Key Words: powder goat milk, fatty acids, water activity

T34 The stability of whipped cream: Effect of emulsifier synergy on partial coalescence and crystallization of milk fat. Y. Wang*[1,2], R. Hartel[1], and L. Zhang[2], [1]*University of Wisconsin–Madison*, *Madison, WI*, [2]*China Agricultural University*, *Beijing, China*.

Whipped cream is a triphasic system where the air is introduced and finally enveloped by a network of partially-coalesced fat globules (FGs). Emulsifiers are an important factor affecting the partial coalescence of FGs, which is critical for the stabilization of whipped cream. Therefore, we investigated the synergy of different emulsifier formulas in the bulk anhydrous milk fat (AMF) system, whipping cream emulsions, as well as how it related to the stabilization of whipped cream. Lactic acid esters of mono-and diglycerides (LACTEM, 0.2 wt.%), Sodium stearyl lactate (SSL, 0.2 wt.%) or Tween80 (0.05 wt.%), either singly or in combination with mono- and diglycerides (MDG, 0.1 wt.%) were used as different emulsifier formulas. The partial coalescence and crystallization profile of bulk AMF blends were examined. Furthermore, the particle size distribution, apparent viscosity, and creaming rate of whipping cream were measured, while the whipping properties and microstructure of whipped cream were analyzed. Results showed that the different emulsifier formulas exhibited various interfacial properties and fat crystallization behavior, affecting the texture of the final whipped cream. Lipophilic-emulsifier formulas accelerated fat crystallization and achieved higher foam firmness, while the MDG had a significant positive effect on the crystallization behavior of bulk AMF blends compared with the minor impact on emulsification. Moreover, the highly viscous whipping creams were obtained by combining additional hydrophilic emulsifiers (SSL or Tween80) could be attributed to FG partial coalescence and smaller particle size. The 4-blend emulsifier formula produced a lower emulsion creaming rate (0.25 ± 0.00 a.u.) and higher overrun (177.03 ± 3.50%). The foam displayed regularly-shaped air bubbles enveloped by fat network consisting of round and well-distributed FGs. Overall, the results provided an interesting starting point for further work on controlling the partial coalescence of FGs and fat crystallization behavior by selecting appropriate emulsifier formula to achieve better texture of whipped cream.

Key Words: whipped cream, milkfat crystallization, stability

T35 Using iso-conversional kinetics to study the effect of α-tocopherol on the oxidation of formulated milk powder. K. A. Alsaleem*[1,2], K. Muthukumarappan[3], and S. I. Martinez-Monteagudo[1], [1]*Dairy and Food Science Department, South Dakota State University*, *Brookings, SD*, [2]*Food Science and Human Nutrition Department, Qassim University*, *Al-Qassim, Saudi Arabia*, [3]*Agricultural and Biosystems Engineering Department, South Dakota State University*, *Brookings, SD*.

Formulated milk powders are used in several applications, including recombined evaporated milk, yogurt, cheese, infant formulas, and beverages. During processing and subsequent storage, the fat in the powder can be oxidized and generate undesirable changes in the final product. The addition of antioxidants is a widespread practice to prevent the oxidation of formulated milk powders. In this work, we studied the effect of α-tocopherol on the kinetic parameters (pre-exponential factor, activation energy, and reaction model) during the oxidation of formulated milk powder. The oxidation kinetics were obtained using thermogravimetrical analysis (TGA) under non-isothermal conditions. Samples were formulated with either α-Tocopherol at 2% (α-Toc), hydrogen peroxide (H_2O_2), and α-Toc+H_2O_2. Then, the formulated samples were oxidized at different heating rates (3, 6, 9, and 12°C min^{-1}) in a temperature range of 100–300°C. In general, the addition of α-Toc delayed the oxidation of formulated milk powders. The lowest value of oxidation onset temperature was obtained for α-Toc, followed by α-Toc+H_2O_2 and H_2O_2, yielding values of 128.2 ± 0.2, 231.8 ± 0.5, and 248.5 ± 0.2°C, respectively. The activation energy values were 58.12 ± 3.21, 46.91 ± 2.75, 69.43 ± 3.05, and 65.89 ± 5.12 kJ mol^{-1} for formulated milk powder, H_2O_2, α-Toc, and α-Toc+H_2O_2, respectively. The reaction model for the different samples was initially screened by fitting 13 different models. The most suitable reaction model to describe the oxidation of formulated milk powders was the Avrami-Erofeev model (A3). The obtained kinetic parameters were interpreted in terms of the oxidation mechanism. The obtained results may enable further process development, design, and optimization.

Key Words: α-tocopherol, isoconversional methods, formulated milk powders

T36 Comparison of milk iodine concentration between retail conventional and organic milk in the United States. M. Ghelichkhan*[2], L. H. P. Silva[2], R. C. R. Tinini[1], J. G. Dessbesell[1], M. A. Zambom[1], and A. F. Brito[2], [1]*Universidade Estadual do Oeste do Paraná*, *Marechal Cândido Rondon, Brazil*, [2]*University of New Hampshire*, *Durham, NH*.

Previous research showed that over 50% of organic dairies in the Northeast and Upper Midwest US feed *Ascophyllum nodosum* meal known to be a rich source of iodine (I). Therefore, milk I concentration (MIC) in retail organic milk may be greater than that of conventional milk. Further, different feeding practices between organic and conventional dairies have been shown to seasonally change MIC, which can be also affected by processing method. However, a comprehensive survey of conventional and organic retail MIC has not been conducted in the US. A total of 299 samples of 2%-reduced fat organic [n = 96; ultra-high temperature (UHT), n = 62; pasteurized, n = 34] and conventional (n = 203; UHT, n = 25; pasteurized, n = 178) milk were purchased in selected grocery stores (n = 73) in June 2017 (summer) and March–April 2018 (spring) from the 11 northeastern states and Washington DC (n = 23 cities visited). No duplicate brand (n = 108) or milk plant (n = 82) was included in the MIC data set. Statistical analyses were done in JMP Pro 15.0.0 using a full factorial ANOVA model that included season (summer vs. spring), production system (conventional vs. organic), and milk processing (UHT vs. pasteurized) as independent variables, as well as interactions. A season by production system interaction ($P < 0.001$) was observed; while conventional milk tended to have greater MIC ($P = 0.09$; 388 ± 17 μg/L) than organic (341 ± 22 μg/L) during the summer, organic milk had increased MIC ($P < 0.01$; 515 ± 21 µg/L) compared with conventional milk (437 ± 16 µg/L) in the spring. This seasonal difference likely results from intake of I-binding goitrogens present in pasture that prevent the transfer of I into milk. UHT milk also had greater MIC ($P < 0.01$; 455 ± 16 µg/L) than pasteurized milk (386 ± 12 µg/L). Note that organic milk had greater MIC during winter than conventional milk and most was UHT processed, indicating that our milk processing data should be interpreted cautiously. Overall, season and processing appear to affect MIC as shown in previous research, and MIC was generally below the 500-µg/L threshold considered safe for consumers.

Key Words: dairy food, milk plant, milk processing

T37 Influence of monk fruit sweetener on the physico-chemical characteristics of camel milk yogurt.

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Monk fruit sweetener contains antioxidants and is a natural zero calorie sweetener with the sweetness from 100 to 250 times that of sucrose. Camel milk has many beneficial nutritional and therapeutic characteristics and is used to treat different human diseases because of the presence of natural bioactive components. Camel milk yogurts with 0, 3.2, 9.6, and 19.2g of the added monk fruit sweetener per 7.57 L of camel milk were prepared and stored at 4°C for 6 weeks. During storage, the pH, titratable acidity, viscosity, and color (L*, a*, b*, C*, and h*) were measured weekly. Three replications were conducted. Data were analyzed using Proc Mixed of the SAS and Bonferroni (Dunn) *t*-test was used to determine significant differences at $P < 0.05$. The presence of monk fruit sweetener significantly decreased the pH and significantly increased the viscosity of the yogurts. Yogurt with 0g of the sweetener had significantly higher pH (4.38 ± 0.10) than the yogurts with 9.6 (4.30 ± 0.07) and 19.2g (4.31 ± 0.10) of monk fruit sweetener. Viscosity of the yogurt with 0g of the sweetener was significantly lower (1394.98 ± 321.83) than the samples with 9.6 (1595.78 ± 219.20) and 19.2g (1646.92 ± 181.50) of the sweetener and no different from the sample with 3.2g of the sweetener (1539.33 ± 131.81). The instrumental color attributes a*, b*, C*, and h* were also significantly affected by the addition of the sweetener. The a* values of yogurts significantly increased, the b*, C*, and h* values of yogurts significantly decreased. The supplementation of yogurt with the sweetener did not affect the titratable acidity and the L* value of yogurt samples. Monk fruit sweetener can be used as a sweetener in the camel milk yogurt production.

Key Words: fermented, yogurt, physico-chemical

T38 Impact of butterfat content and composition on the quality of laminated pastries.

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Bakers (professional and amateur) often choose butters with higher butterfat content (>82% butterfat) and the industry is responding with a variety of options that deliver certain butterfat percentages (up to 85% butterfat). Higher butterfat products are more challenging to manufacture; however, they also demand a higher price point making their production appealing to processors. The objective of this study was to associate butter characteristics (butterfat, fatty acid profile, etc) with dough performance and finished quality of laminated pastries (height, weight). Commercial butters (n = 14) were sourced from local retailers and used as the fat component in a standardized croissant dough. The dough was laminated and sheeted at approximately 12°C using the Rondo SSO615 Ecomat Floor Model Sheeter. Dough was cut and formed into croissants, proofed at 30°C for 90 min at 80% RH, and baked at 196°C for 15 min in a rotating convection oven. The butterfat content, fatty acid profiles, and melting profiles of each butter were characterized using Mojonnier method AOAC 922.06, GC-FAME, and differential scanning calorimetry, respectively. Heights and weights and crumb characteristics of laminated and baked pastries were measured. The majority of commercial butters performed acceptably (no cracks or tears) during lamination and produced finished croissants of good quality; however, one dough was noticeably sticky and difficult to handle. Four of the commercial butters produced finished pastry of low baked height, likely a function of poor rise during proofing. Butterfat content was not found to be responsible for the difference in dough quality ($P > 0.05$). Instead increased unsaturated fat content (highly dependent on C18:1; $P = 0.04$) was associated with decreased baked pastry height. None of the other measured characteristics were associated with finished product quality.

Key Words: butter, croissant, baking

T39 Influence of cell surface properties on the adhesion potential of environmental *Listeria* isolates to dairy floors.

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The prevalence of *Listeria* in dairy manufacturing environment poses a cross-contamination risk. The current study compares 3 *Listeria* isolates from dairy plants; *L. monocytogenes* (*Lm*), *L innocua* (*Li*), and *L. welshimeri* (*Lw*), for their cell surface properties and adhesion on different floor types; clay brick, poured concrete, and grout. For evaluating cell attachment, different floor chips (1x1cm^2) were immersed in sterile distilled water, containing 6 logs/mL of the individual *Listeria* strain, and held for 1h at 22.4°C in a shaker incubator. The attached cells were retrieved from floor chips using 3M sponge sticks and plated on brain heart infusion agar. The counts obtained were reported as cfu/cm^2. The cell surface hydrophobicity was determined by the hexadecane method. The absorbance of aqueous layers was measured, and % hydrophobicity was calculated. The zeta potential was determined by the Zeta sizer Nano series instrument (Malvern Panalytical, UK). In addition, the contact angle of HPLC water on the dairy floor types was measured using the Sessile Drop device. For each of the analyses, 3 trials were conducted, with samples drawn in triplicates, and the means were compared by ANOVA. The results from the attachment study indicated the highest adhesion (log cfu/cm^2) of all 3 isolates on poured concrete (*Lm* 3.54 ± 0.04, *Li* 3.27 ± 0.16 and *Lw* 3.70 ± 0.24), as compared with grout (*Lm* 3.28 ± 0.51, *Li* 2.67 ± 0.41 and *Lw* 2.71 ± 0.53), and clay brick (*Lm* 2.91 ± 0.16, *Li* 2.73 ± 0.04 and *Lw* 2.89 ± 0.40). The water contact angles on all 3 surfaces were less than 90° (clay brick 43.3 ± 0.78, poured concrete 47.9 ± 3.23 and grout 64.5 ± 2.85) indicating them to be hydrophilic and thus supporting the attachment. Of the 3 isolates, *Lm* and *Lw* resulted in a greater attachment than *Li*, which was also supported by their respective hydrophobicity values (20.13%,21.15%, and 13.88%). The zeta potential values, however, were similar (−15.8 mV, −16.2 mV and −15.5 mv, respectively) and did not appear to influence attachment. The study provides critical information for selecting the type of floors that may reduce the colonization and biofilm formation by the environmental *Listeria* in dairy plants.

Key Words: dairy floors, attachment, *Listeria*

T40 Inhibition of *Listeria monocytogenes* by lactose oxidase in UHT skim milk.

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Listeria monocytogenes is a ubiquitous pathogen that grows at refrigeration temperature and can cause mortality in immunocompromised individuals. Hispanic-style fresh cheeses are susceptible to the growth of *L. monocytogenes* due to their high water activity, low salt content, and near neutral pH. Traditionally, these cheeses are produced using raw milk which increases the risk of *L. monocytogenes* contamination due to the lack of a pasteurization step. The use of antimicrobials in raw milk is a potential way to control *L. monocytogenes* growth in processes without a thermal kill step. Microbial-based enzymes offer a clean-label approach for control of *L. monocytogenes.* Lactose oxidase (LO) is a microbial-derived enzyme with antimicrobial activity. It oxidizes lactose into lactobionic acid and generates hydrogen peroxide. This study investigated the utilization of LO for the control of *L. monocytogenes* growth in UHT skim milk as a model system for future applications in raw milk. Three concentrations of LO, 0.006, 0.012, and 0.12 g/L, were evaluated for their ability to inhibit *L. monocytogenes* growth at 6°C over 21 d. UHT skim milk samples containing these LO concentrations, and a control with no LO, were inoculated with either 4 log cfu/mL or 2 log cfu/mL of a 5-strain cocktail of *L. monocytogenes*. Samples were enumerated for growth of *L. monocytogenes* on d 0, 2, 4, 7, 14, and 21. Analysis of Variance and Tukey's Honest Significant Difference tests were performed individually for each time point and log differences between the control and treatments were determined. By d 2, all treatments showed significant differences ($P < 0.001$) from the control in both challenge experiments. Significant differences between treatments and the control continued throughout the 21-d trials at each time point. By d 21 the control grew to over 7 log cfu/mL while all treatments remained at 1 log cfu/mL. These results suggest that LO is a potential control strategy for *L. monocytogenes* growth in milk, and future studies will investigate its antimicrobial efficacy in raw milk and cheese applications.

Key Words: *Listeria*, enzymatic preservation, milk

T41 ***Bacillus cereus*** group species isolated from dairy products and dairy environments are not appropriate for use in inoculated-milk human sensory studies.

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Bacterial spoilage limits the refrigerated shelf-life of conventionally pasteurized fluid milk, but the specific metabolic processes that result in many types of milk spoilage are not well-understood. To address this deficiency, bacterial strains that reliably and reproducibly generate specific sensory defects in fluid milk must first be identified. Human sensory panels are the current standard for sensory defect identification, but the safety of panelists must be ensured if they are to taste intentionally inoculated milk. This raises a particular concern for the *Bacillus cereus* group, members of which are associated with both milk spoilage and foodborne illness. We used BTyper, a computational tool for virulence-based classification of *B. cereus* group isolates, to examine the whole genome assemblies of 37 dairy-associated *Bacillus* spp. for 4 common virulence factors associated with enteric illness: cereulide, hemolysin BL, non-hemolytic enterotoxin, and cytotoxin K. Genes encoding all 4 of these factors were detected among the 37 *Bacillus* spp. isolate assemblies. Specifically, a gene necessary for cereulide production (cesC) was detected in 4 isolates, the 4-gene operon necessary for hemolysin BL production (hblCDAB) was detected in 27 isolates, the 3-gene operon necessary for non-hemolytic enterotoxin production (nheABC) was detected in 36 isolates, and the gene necessary for cytotoxin K-2 production was detected in 16 isolates. In addition to these virulence factors, genes encoding resistance to β-lactams, fosfomycin, or vancomycin were detected in each of the 37 *Bacillus* spp. genomes. In contrast, none of these 4 virulence factors and very few antimicrobial resistance genes were detected in 61 whole genome assemblies of *Paenibacillus* spp. or in 5 whole genome assemblies of *Viridibacillus* spp. Based on these findings, we were unable to identify any *B. cereus* group isolates that we judged sufficiently lacking in potential hazards to justify their intentional exposure to a human sensory panel. Hence, other methods must be used to assess the spoilage potential of *Bacillus* spp.

T42 Effect of cavitation and nanofiltration temperature on the functional properties of milk protein concentrate (MPC80).

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Nanofiltration (NF) is typically utilized during milk protein concentrate (MPC) manufacture to concentrate ultrafiltration retentate (feed) before drying. In this study 3 replicates of 4 different NF treatments were assessed for their impact on powder characteristics and functionality. The NF (200 Da MWCO) treatments utilized were: NF at 22°C (NF22); NF at 50°C (NF50); hydrodynamic cavitation (HC) before NF at 22°C (HCNF22); and HC before NF at 50°C (HCNF50). The total protein (TP) to total solids ratio of all retentates was very close to the feed (0.8) ($P > 0.05$). Non-casein nitrogen (NCN) and non-protein nitrogen (NPN) in the NF retentates were 0.57, 0.79, 0.58 and 0.72% and 0.12, 0.11, 0.18 and 0.20% respectively for the NF22, NF50, HCNF22, and HCNF50 treatments. Both high temperature and HC did not have a significant ($P > 0.05$) impact on NCN and NPN. Protein fractions such as β-CSN and α-S1 made up about 70% of the TP and were statistically similar ($P > 0.05$) in all the treatments. γ-CSN was increased significantly ($P < 0.05$) for NF50 and HCNF50 treatments and the rennet coagulation time (RCT) increased significantly ($P < 0.05$) in NF50 and HCNF50 treatments. RCT without $CaCl_2$ was 105, 125, 100 and 121 min and with 0.1% $CaCl_2$ was 14, 21, 13 and 18 min respectively for the NF22, NF50, HCNF22, and HCNF50 treatments. There was not a significant ($P > 0.05$) difference in the Ca and P of all NF treatments. The relative dissolution index decreased significantly ($P < 0.05$) in the 50°C treatments but increased significantly ($P < 0.05$) in HCNF22. Both loose and tapped density were highest for the HCNF50 treatment (286 and 366 Kg/m^3 respectively). However, HC also significantly ($P < 0.05$) increased the tapped density of MPC powder. Neither HC nor high temperature NF significantly ($P > 0.05$) affected the diameter, circularity, elongation, solidity and convexity of the powder particles. The standard plate count of the MPC powder was significantly ($P < 0.05$) higher for the NF50 and HCNF50 treatments. This study determined that NF temperature and HC have important impacts on the properties of MPC and can be utilized to adjust the functional characteristics of MPC powders.

Key Words: milk protein concentrate, hydrodynamic cavitation, nanofiltration

T43 Development and characterization of whey-buttermilk fermented beverages with Gabiroba pulp (*Campomanesia xanthocarpa*).

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The use of dairy coproducts such as whey and buttermilk is a high-value food production strategy. This work aims to develop and to characterize whey-buttermilk fermented beverages with gabiroba pulp (*Campomanesia xanthocarpa*). Were produced 13 formulations according to the simplex-centroid mixture, designed with different concentrations of whey and buttermilk between 0 and 44%, UHT milk between 40 and 56% and pulp between 10 and 20%. Batches of 20 L of milk, whey and buttermilk were heated at 85°C/5 min, cooled at 42°C and fermented with 2.5% of starter culture YoFlex Harmony 1.0 (Chr. Hansen, Denmark) for 3h until reach pH 4.85. Fermented beverages were cooled, added of gabiroba pulp, packaging in glass bottles and then stored at 5°C. Physicochemical and microbiological characteristics were determined (n = 3) by official methods including analysis of lactic acid bacteria (LAB) by MRS agar at 37°C/72h, coliforms by BGBB 2% broth at 35°C/48 h and EC broth at 45°C/24 h. Presence of *Salmonella* sp. was evaluated using buffered peptone water at 37°C/18 h, RVS broth at 41.5°C/24 h, MKTTn at 37°C/24 h and XLD agar and BS agar at 37°C/24 h. Results were evaluated by ANOVA and mean values were compared by Scott-Knott's test ($P < 0.05$). All beverages met the expected characteristics for quality and food safety. They presented pH between 4.34 and 4.76, acidity between 0.34 and 0.56%, moisture between 79.51 and 83.05%, ash between 0.33 and 0.50%, protein between 1.49 and 2.24%, casein between 1.14 and 1.88%, whey proteins between 0.02 and 0.48%, lipids between 1.09 and 1.78% and carbohydrates between 13.53 and 15.96%. All beverages presented a significant difference ($P < 0.05$) for physicochemical parameters. This result was due to different concentrations of ingredients. A higher concentration of milk results in higher protein and lipid content, and a higher concentration of buttermilk results in higher moisture. All the beverages presented LAB above 6.0 $\log^{10}$ CFU/mL for 7 weeks and 6 of them for 13 weeks. The use of whey and buttermilk to produce fermented beverages is a suitable way of making functional and sustainable dairy products.

Key Words: functional, coproducts, innovation

Extension Education 1

T44 Texas dairy industry assessment of research, education, and service importance of the Southwest Regional Dairy Center. B. W. Jones*[1,2], [1]*Tarleton State University*, *Stephenville, TX*, [2]*Texas A&M AgriLife Research*, *Stephenville, TX.*

The Southwest Regional Dairy Center (SWRDC) is the Texas A&M University system wide dairy located in Stephenville, TX, and is administratively managed through Tarleton State University. The SWRDC opened its doors in 2011 and its mission is to provide enabling infrastructure support for teaching, research, and service/outreach programs to meet the needs of higher education, the dairy industry, and society in Texas and the southwest. The objective of this research was to determine future directions the SWRDC should take and what the SWRDC could improve upon. In October 2019, a Qualtrics survey (Qualtrics LLC., Provo, Utah) was distributed to the Texas Association of Dairymen and extension agents to further distribute to dairy producers and industry representatives. The survey asked respondents to rank satisfaction with tours of the SWRDC from 1 to 7 (1 = extremely satisfied, 7 = extremely dissatisfied) and to rank community outreach events, research activities, extension events, and types of research from least important to most important. Twenty-eight producers and dairy industry representatives responded. Statistical analyses were completed on the survey responses using SAS (Version 9.4, SAS Institute, Inc., Cary, NC). The MEANS procedure calculated satisfaction with tours. The FREQ procedure was used to determine the highest ranked response for community outreach events, research activities, extension events, and types of research that should be conducted. On average respondents were extremely satisfied with tours of the SWRDC (1.77 ± 1.30; respondent range was 1 to 4). The respondents' highest ranked community outreach event, research activity, extension event, and type of research were, breakfast on the farm, research showcase, demonstration days with continuing education credits offered, and ruminant nutrition, respectively. Survey findings were informative and help inform the SWRDC administration of future directions the center should take in research, education, and service activities.

Key Words: extension, education, service

T45 Wisconsin farmer-reported housing and milk-feeding practices for preweaned dairy calves. J. Van Os*[1], C. Winder[2], M. Akins[1], T. Kohlman[3], T. Ollivett[4], H. Schlesser[3], B. Schley[3], S. Stuttgen[3], and J. Versweyveld[3], [1]*Department of Dairy Science, University of Wisconsin-Madison*, *Madison, WI*, [2]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*, [3]*Division of Extension, University of Wisconsin-Madison*, *Madison, WI*, [4]*School of Veterinary Medicine, University of Wisconsin-Madison*, *Madison, WI.*

Research has indicated many benefits of socially rearing pre-weaned calves, but little is known about current industry practices. Our objective was to characterize calf-rearing practices in WI. A Qualtrics survey was distributed to dairy farmers and calf raisers. On average, WI respondents (n = 202) had 103 milk-fed calves and 469 milking cows. Most farms (164, 81%) housed calves only individually, although 38 (19%) housed some heifers socially. Of farms using social housing, 23 (61%) kept calves in groups of 2–8, and 15 (39%) had larger groups. The most common housing was indoor pens with manual feeding (17 farms, 45%); 13 farms (34%) had indoor pens with automatic feeders, and 8 farms (21%) kept some of their heifers outside in "super hutches" or adjoined hutches. The most common maximum age range within groups was <1 wk (18 farms, 47%), with most calves entering groups at ≤14 d old (24 farms, 63%). Most farms with social housing (74%) fed milk or replacer through a teat (bottle, teat bucket, or automatic feeder) for most of the milk-feeding stage, but only 24% of farms with only individual housing fed through a teat. Four-week old calves were fed milk or replacer at a volume of 6.9 ± 2.4 vs. 7.4 ± 2.3 L/d (mean ± SD) on farms housing their calves only individually vs. using social groups, respectively (>97% response rate). In rating the level of satisfaction with their calves' growth performance, 87 vs. 86% of those using only individual vs. some social housing indicated they were somewhat or extremely satisfied (>97% response rate). In rating the level of satisfaction with their calves' health, 84 vs. 89% of those using only individual vs. some social housing indicated they were somewhat or extremely satisfied (100% response rate). Of the farmers using only individual housing, 64 (39%) indicated they were interested in learning more about social rearing. These results demonstrate that many WI farmers are managing pre-weaned calves in social groups using a range of practices, and with the majority expressing satisfaction with the outcomes. This information can be used to better target future research and extension education programs.

Key Words: survey, welfare, heifers

T46 Use of dairy advisory teams as a tool for improvement. L. A. Holden*, *The Pennsylvania State University*, *University Park, PA.*

The complexity of dairy farm businesses mean that owners need to have expertise in many different areas, including crop and dairy production, herd health, workforce, financial and business management. Most dairy producers rely heavily on farm advisors for information, advice and help with problem solving. Sometimes that advice and help can result in conflicting information. Dairy advisory teams are one business tool that allow producers to bring all their advisors together on a regular basis to sort conflicting information and move forward successfully. The objective of this project was to evaluate the impact of dairy advisory teams on farms and the measure the extent of involvement from farm advisors. Written surveys were mailed to 104 dairy producers using teams and 262 farm advisors working with at least 1 of the 104 teams. Response rate was 73 of 104 or 70.2% for dairy producers and 132 or 262 or 50.4% for farm advisors. After the first year of using a team, dairy producers (n-58) indicated that the success rate for areas targeted for improvement was 81.1% for record keeping and use of farm records for decision making, 68.3% for increased milk production and 66% for improved nutrition or reduction in feed costs. Some other targeted areas had less than 60% rate of success. Before the use of a dairy advisory team, only 15.5% of dairy producers felt that regular communication with advisors was important compared with 63.2% after using the team. Farm advisor responses indicated that 64% of their teams had 5 to 6 members, and a majority (61.40%) of advisors spent between 10 and 40 h per year working with a team. When asked about satisfaction with milk production and cash flow, nearly half of the dairy producers were "not satisfied" before team use but less than 10% indicated "not satisfied" after use of the team. Farm advisor satisfaction with communication increased from 23.1% before the team to 82.9% after the use of the team. Well managed dairy advisory teams can be a sound business tool for improving communication among farm advisors and for making positive on-farm changes in targeted areas.

Key Words: team problem solving, dairy management, farm advisors

T47 Evaluation of trainings provided to Central Texas dairy workers. J. A. Garcia Buitrago*[1], G. R. Hagevoort[1], J. Spencer[2], and J. Pineiro[2], [1]*New Mexico State University, Agricultural Science Center at Clovis*, *Clovis, NM*, [2]*Texas A&M University, Texas AgriLife Extension*, *College Station, TX.*

A close relationship has been reported between the work performed by workers who have received training in animal handling, animal welfare, reduction of occupational risks and the increase in productivity of dairy farms. Cattle behavior toward people depends on instinctive actions, reactions to external stimuli and acquired conducts, their knowledge through training can help improve job performance. Between 2015 and 2018 New Mexico State University and Texas A&M University dairy extension specialists conducted animal handling trainings with dairy workers from Central Texas dairy farms with the goal to affect their animal handling skills. Three-hour training sessions conducted in both Spanish and English using audiovisual presentations were followed by live demonstrations with dairy cows. The training included 5 conceptual elements: how do cows perceive their surroundings, blind spots, flight zone, point of balance and moving animals. To determine the level of comprehension of the trainings, 104 participants were asked to answer 10 objective questions about the concepts of animal behavior and handling, in identical tests conducted before and after the training session. The tests were scored according to the number of correct answers, their means were compared using a *t*-test, and the effects of age, educational level, language and gender of participants and training year were analyzed using an ANOVA. The results show a statistical difference ($P < 0.05$) in the means of the tests before and after training. The average score was 2.49 points higher in the post-training test and varied between a range of 2.23 to 2.75 (90% confidence interval). No significant differences were found due to effects considered. Results showed an improvement in the understanding of the basic concepts of animal handling, regardless of the age, sex, language and educational level of the participants, and training year. Training in basic animal handling concepts could help improve the work quality, animal welfare and dairies productivity.

Key Words: animal handling, dairy, training

Forages and Pastures 1

T48 Microbial community and fermentation dynamics of corn silage prepared with heat-resistant lactic acid bacteria in hot environment.

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Abstract With global warming, high temperatures become an important factor that influence silage fermentation process in the world, especially in the tropical and sub-tropical areas. To develop a silage fermentation technique to adapt to global climate changes, the microbial community and fermentation dynamics of corn silage prepared with a heat resistant lactic acid bacterium under different high temperature conditions were studied. Corn was ensiled in laboratory silo, with and without 2 selected strains *Lactobacillus salivarius* LS358 and *L. rhamnosus* LR753, 2 type strains *L. salivarius* ATCC 11741^T and *L. rhamnosus* ATCC 7469^T, and a commercial inoculant *L. plantarum*. Every treatment used 3 mini PVC silos (3 kg of fresh forage, density as 250 kg/m^3) as replicates. The ensiling temperatures were designed at 30°C as control and at 45°C as high temperature, and the sampling took place after 0, 3, 7, 14 and 60 d of fermentation. During ensiling at 30°C, the dominant bacteria genera gradually changed from *Pediococcus* and *Lactobacillus* to *Lactobacillus* in silages. When silage stored at 45°C, bacterial community became more complex and fragmented after 7 d of ensiling. *Pectobacterium*, *Brevundimonas*, and *Bosea* spp. became prevalent at terminal fermentation. High temperature significantly caused a shift from a homofermentative to a heterofermentative type of LAB population in corn silage. Notably, the presence of *Acetobacter* was associated with silage fermentation at high temperature. The higher ($P < 0.05$) pH values and DM losses were observed in the silages stored at 45°C than those at 30°C, and the lower ($P < 0.05$) ratio of lactic acid/ acetic acid were found in silages inoculated with strains LS358 and LR753 at 30°C. Generally, exogenous heterofermentative *L. rhamnosus* 753 had potential ability to survive at 45°C during first 2 weeks of ensiling, reduced pH and produced more acetic acid content. Due to high temperature silage is an uncontrolled fermentation process, analysis of bacterial community of silage by next-generation sequencing (NGS) can be used for optimization of the ensiling process and heat resistant strain *L. rhamnosus* 753 show potential ability to improve silage fermentation in tropics and subtropics.

Key Words: bacteria community, high temperature, corn silage

T49 Effect of microbial inoculation and storage length on fermentation profile, aerobic stability, and nutrient composition of whole-plant sorghum.

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The objective of the study was to determine the effects of storage length and the addition of a heterofermentative microbial inoculant containing a mixture of *Lactobacillus diolivorans, Lactobacillus buchneri,* and *Lactobacillus plantarum* in sorghum silage on fermentation profile, aerobic stability and nutrient composition. Forage from a brown midrib sorghum hybrid was harvested at approximately 20% DM and inoculants (distilled water or heterofermentative inoculant) were applied to fresh forage. Inoculant was applied at a rate of 300,000 cfu/g of fresh forage based on bacterial counts previously conducted. Treatments were ensiled in quadruplicates in 20-L laboratory silos and opened after 14, 28 or 56 d of fermentation. Data were analyzed as a completely randomized design with a 2 × 3 factorial arrangement using Proc Glimmix of SAS and significance was declared at $P < 0.05$. An interaction among treatments was observed for acetic acid concentration ($P = 0.006$), with inoculated silage being greater than control and concentration increasing with storage length. Interactions were also observed for mold, DM content, lactic acid, and 1-propanol. Inoculation increased production of 1, 2-propanediol (0.52 vs 0.00% of DM; $P = 0.01$), 2, 3-butanediol (0.23 vs 0.00% of DM; $P < 0.001$) and ethanol (1.36 vs 0.74% of DM; $P = 0.002$). Conversely, inoculation decreased pH (3.86 vs 3.95; $P < 0.001$) and ammonia production (0.42 vs 0.51% of DM; $P = 0.006$). Additionally, a tendency was observed for inoculation to increase aerobic stability (122.19 vs 82.23 h; $P = 0.09$). Total acid content was affected by both inoculation and storage length ($P = 0.008$ and $P < 0.001$, respectively) with inoculant increasing acid production (11.46 vs 10.60% of DM) and 56 d having the greatest acid concentration (12.46 vs 10.32%of DM). There was no effect of inoculation or storage length on yeast or propionic acid concentration. These results suggest that inoculation with this heterofermentative microbial inoculant positively affect fermentation under short-term storage conditions.

Key Words: *Lactobacillus diolivorans*, *Lactobacillus buchneri*, sorghum silage

T50 Effect of inoculant dose and time of ensiling on the fermentation and aerobic stability of corn silage.

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We evaluated the effect of an inoculant on the fermentation and aerobic stability of corn silage. Fresh whole-plant corn (Master's Choice 6150) was harvested (19-mm chop length) from 2 separate fields (on different days, 42.25 and 37.68% DM, respectively) with a pull behind chopper equipped with a kernel processor (roll gap setting 1.5 mm). Forage from each field was untreated (CTRL) or treated with a low level of SilagePRoB (SP) (SP-L; American Farm Products, Saline, MI; application rate: 200,000 cfu of /g of *L. buchneri* (LB)/ g of fresh weight and 50,000 cfu of *Pediococcus pentosaceus*, 50,000 cfu of *P. acidilactici* and isolated enzymes from *Aspergillus oryzae* and *Bacillus subtilis* or, a high level of SP (SP-H; differing from SP-L in that the application rate for LB was 400,000 cfu/g). Treatments were applied as individual replicates in quintuplicate and ensiled in 7.5L bucket silos (density of 240 kg of DM/m^3) at 21? for 30 and 90 d. Data were analyzed by ANOVA as a completely randomized design in a factorial arrangement of treatments (2 d of ensiling × 3 inoculation treatments × 2 fields) using JMP with significance declared at $P < 0.05$. At 30 d the concentration of acetic was higher only for SP-H compared with CTRL, but it was higher for SP-L and SP-H compared with CTRL at 90 d. Both levels of inoculant increased concentrations of 1,2 propanediol at both ensiling times. Inoculation increased the numbers of lactic acid bacteria compared with CTRL. There was a day x treatment interaction for aerobic stability and yeasts. At 30 d, only SP-H resulted in fewer yeasts and improved stability when compared with CTRL. However, at 90 d, both levels of inoculant markedly decreased the numbers of yeasts (inoculated = <log 1 vs. CTRL = 4.85 log cfu/g) and improved aerobic stability (inoculated average of 225 h) compared with CTRL (44 h). These data show that when using the strain of LB in SP, a higher application rate was needed for a small improvement in aerobic stability after 30 d of ensiling, but that either level of inoculation was markedly efficacious when silage fermented for 90 d.

Key Words: corn silage, microbial inoculant, aerobic stability

T51 Effects of *Lactobacillus hilgardii* 4785 and *L. buchneri* 40788 on the bacterial community of high-moisture corn.

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ware, *Newark, DE*, [2]*Lallemand Specialties Inc*, *Milwaukee, WI.*

We evaluated the effect of 2 additives (Lallemand Animal Nutrition, Milwaukee, WI) on the bacterial community dynamics of high moisture corn (HMC) during ensiling. The HMC (71% DM) was untreated (CTR), treated with *L. buchneri* NCIMB 40788 (LB; 600,000 cfu/g of fresh wt.), treated with *L. hilgardii* CNCM I-4785 (LH; 600,000 cfu/g), or treated with LB + LH (LBLH; 300,000 cfu of each/g). Three individually replicated lab silos (7.5 L) for each treatment were packed (669kg DM/m^3) and ensiled for 10, 30, and 92 d between 21 and 23°C before being sampled for the analysis of the bacterial community by the sequencing of the 16S rRNA V3-V5 region using Illumina MiSeq. Relative abundance (RA) data were analyzed as a 4 × 3 factorial design with the fixed effects of additive, length of ensiling, and their interaction using JMP (SAS Institute Inc.). The distance matrix was calculated using Phyloseq (McMurdie and Holmes, 2013). The untreated grains before ensiling formed a cluster separated from ensiled samples and CTR silages formed clusters separated from treated silages. No separation among LB, LH, and LBLH was observed but treated HMC ensiled for 92 d clustered apart from treated HMC ensiled for 10 and 30 d. The LB, LH, and LBLH HMC had similar RAs of *Lactobacillaceae*, *Enterobacteriaceae*, *Leuconostocaceae*, and *Streptococcaceae.* Treated HMC had a higher ($P < 0.05$) *Lactobacillaceae* RA than CTR (71.20 vs. 19.09%) at all lengths of storage and CTR had a higher ($P < 0.05$) *Enterobacteriaceae* RA than treated HMC (23.72 vs. 4.37%). At 10 and 30 d, CTR had a higher ($P < 0.05$) RA of *Leuconostocaceae* (58.46 vs. 16.20%) and *Streptococcaceae* (4.39 vs. 1.51%) than treated HMC, but at 90 d the RA of those families were similar among treatments (32.22 and 0.68%, respectively). *Lactobacillaceae* dominance on treated HMC explained their higher aerobically stability compared with CTR, previously observed by Polukis et al. (2016). The additives increased the RA of *Lactobacillaceae* and reduced the RA of other families, such as *Enterobacteriaceae*, which comprises strains that can reduce the nutritive value and hygienic-sanitary quality of HMC.

Key Words: bacterial community, *Lactobacillus hilgardii*, silage

T52 Evaluation of an enzymatic lignocellulolytic complex on corn silage fermentation profile. B. C. Agustinho*[1,2], L. M. Zeoula[1], L. F. Ferraretto[2], H. F. Monteiro[2], M. R. Pupo[3], L. G. Ghizzi[4], M. C. N. Agarussi[2], C. Heinzen Junior[2], J. R. Vinyard[2], S. L. Bennett[2], and A. P. Faciola[2], [1]*Universidade Estadual de Maringá*, *Maringá, Paraná, Brazil*, [2]*University of Florida*, *Gainesville, FL*, [3]*Escola Superior de Agricultura*, *Piracicaba, São Paulo, Brazil*, [4]*Universidade de São Paulo*, *Pirassununga, São Paulo, Brazil.*

Lignin, present in plants cell wall, reduces cellulose and hemicellulose degradation by ruminal microorganisms. However, some organisms produce enzymes capable of degrading lignin and cellulose that can modify the chemical composition, and consequently, the silage fermentation profile. Therefore, the objective of this study was to evaluate the effects of the addition of an enzymatic lignocellulolytic complex over time on fermentation profile in corn silage. The enzymatic lignocellulolytic complex consisted of laccase, Mn peroxidase, lignin peroxidase, and cellulase and it was produced in the lab, under controlled conditions and the enzymatic concentration and activities were determined. Experimental design consisted of 30 treatments (5 enzymatic levels × 6 opening time points), with 4 mini-silos per treatment. The levels tested were 0, 8.7, 17.4, 26.1, and 34.8 mg enzyme/kg of fresh matter. Enzymatic complex was added and manually mixed in the chopped whole plant corn before ensiling. Samples were ensiled in vacuum-sealed bags and opened at 0, 1, 2, 3, 7, and 30 d after ensiling. The samples were used to make the extract to determine pH, NH_3-N, and organic acids. Data were analyzed as a completely randomized design in a 5 × 6 factorial arrangement of treatments using the GLIMMIX procedure in SAS. There was no interaction ($P > 0.05$) between enzymatic levels and opening time points for the parameters evaluated. Also, there was no difference ($P > 0.05$) in total acids concentration in the silage among the tested enzymatic levels, as well as in the molar proportion of the main acids (lactic, propionic, acetic, and butyric acids), consequently, no difference was observed in the pH. The NH_3-N concentration was also not affected ($P > 0.05$) by the enzymes. However, the pH decreased and the total acids and NH_3-N concentration increased over time ($P < 0.01$). Our results indicate that the addition of this enzymatic lignocellulolytic complex during ensiling did not affect corn silage fermentation profile.

Key Words: fiber, fibrolytic enzymes, lignin

T53 Effect of storage length and microbial inoculation on fermentation, dry matter loss and aerobic stability of wet brewer's grains ensiled with increasing amounts of corn grain. C. Heinzen Jr.*, M. Agarussi, C. L. McCary, M. R. Pupo, L. G. Ghizzi, E. C. Diepersloot, B. A. Saylor, and L. F. Ferraretto, *Department of Animal Sciences, University of Florida*, *Gainesville, FL.*

The objective of this study was to evaluate the effects of microbial inoculation and storage length on the fermentation profile, dry matter losses and aerobic stability of wet brewers grains (WBG) ensiled with increasing amounts of dry ground corn (DGC). Pure WBG (21% of DM) and 3 mixtures (M) of WBG and DGC were prepared targeting for a dry matter content of 35% (RC35), 50% (RC50) or 65% (RC65). Distilled water (CON) or 1 of 2 microbial inoculants (I) were applied, a homofermentative with *Pediococcus pentosaceus* at 1×10^5 and *Propionibacterium freudenreichii* at 2×10^4 cfu/g of fresh forage (PPPF), or a heterofermentative with *Lactobacillus buchneri* at 7.5×10^5 and *Pediococcus pentosaceus* at 1×10^5 cfu/g of fresh forage (LBPP). Mixtures were ensiled in laboratory silos in quadruplicates and opened after 2 storage lengths (SL), 30 or 60 d. Data were analyzed as a completely randomized design with a 4 × 3 × 2 factorial arrangement using Proc Glimmix of SAS. Butyric acid concentration decreased ($P < 0.04$) with corn addition or inoculation with LBPP. Mixtures with greater amounts of DGC (RC50 and RC60) reduced ($P < 0.01$) DM losses, regardless of inoculation and storage length. Adding corn to WBG reduced pH, regardless of microbial inoculation treatment. Lactic acid concentration was increased ($P \leq 0.05$) with intermediate addition of corn (RC35 and RC50). Acetic acid concentration did not differ among inoculation treatments when corn was added to the mixtures; however LBPP reduced ($P < 0.04$) acetic acid in pure WBG. Moreover, LBPP increased aerobic stability only for the RC65 mixture. Silos treated with LBPP also had greater 1,2-propanediol concentration when compared with other inoculants in RC50 and RC65. These results suggest that the addition of DGC to WBG improves fermentation and decreases DM losses, whereas LBPP inoculation improves aerobic stability in mixtures containing more than 65% of DM.

Key Words: brewer's grain, inoculants, aerobic stability

T54 Fermentation and protein evaluation of 12 binary legume-grass mixtures ensiled during multiple small-plot harvests using vacuum-bag mini-silos. A. Wilder* and S. Bosworth, *University of Vermont*, *Burlington, VT.*

Perennial legumes and grasses are commonly grown in association for forage production in temperate regions of the world. Although much of this forage is preserved as fermented feed, little is known about how legume and grass species influence the fermentation and protein quality of legume-grass silages. To address this, binary mixtures of legumes (alfalfa, red clover, and birdsfoot trefoil) and grasses (timothy, tall fescue, meadow fescue, and perennial ryegrass) from small plots were artificially wilted to 45% DM and ensiled across 6 cuttings over 2 years. The grass proportion was measured in the field and used as a covariate for analysis and additional measurements were taken on vacuum-bag mini-silos to determine volumetric fermentation expansion and silage pH. Near infrared reflectance spectroscopy (NIRS) was used to determine crude protein, soluble protein (fractions A and B_1), insoluble degradable protein (fractions B_2 and B_3), and degradable protein (fractions A, B_1, B_2 and B_3). A split-plot analysis of covariance (ANCOVA) was used to analyze each cut and significance ($\alpha = 0.05$) varied by cut for each parameter. Across all cuttings, legume-grass mixtures containing birdsfoot trefoil or red clover generally proportioned significantly more crude protein as insoluble degradable protein (fractions B_2 and B_3) and less crude protein as rapidly degradable soluble protein (fractions A and B_1) compared with alfalfa mixtures. While grass species generally had little effect on silage protein fractions, tall fescue inclusion resulted in elevated soluble protein and reduced insoluble degradable protein in some cuttings. Mini-silos containing alfalfa-grass mixtures sometimes showed considerable expansion (up to nearly 200% of their original size), resulting in significant legume effects for 1st cut ($P = 0.0143$) and 3rd cut ($P = 0.0003$) ensilings. Significantly lower expansion was observed in red clover and birdsfoot trefoil mixtures in these cases.

Key Words: legume-grass mixtures, protein fractions, silage fermentation

T55 Effects of sorghum hybrid, microbial inoculation, and storage length on fermentation profile, ruminal starch disappearance and aerobic stability of sorghum silage.

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Interest in growing sorghum for silage has increased, especially in areas where water supply is an issue for plant growth. Therefore, the objective of this study was to evaluate the effects of 2 sorghum (*Sorghum bicolor* L.) hybrids (forage vs. sudan), microbial inoculation and storage length on the fermentation profile, DM loss, aerobic stability and ruminal in situ starch disappearance of whole-plant sorghum silage. Whole-plant sorghum (27.9 ± 37% of DM for forage and 23.2 ± 29% of DM for sudan) was ensiled in quintuplicate mini-silo pouches bags untreated (CON) or with the following treatments: *Lactobacillus plantarum* CH6072, *Lactobacillus plantarum* LSI, and *Pediococcus pentosaceus* P6 at 1×10^5 cfu/g of fresh forage (LPPP); *Lactobacillus buchneri* LB1819 and *Lactococcus lactis* O224 at 1.5×10^5 cfu/g (LBLL). Silos were allowed to ferment for 0, 15, 30 or 90 d. Data were analyzed as a completely randomized design in 2 × 3 × 4 factorial arrangement of treatments using PROC GLIMMIX of SAS. The model included sorghum cultivar, microbial inoculation, storage length, and their interactions as fixed effects. Lactic acid and total acids were greatest in sudan sorghum silage ($P < 0.01$). Ammonia-N (as % of total N) was greatest with sorghum sudan ($P < 0.01$). The acetic acid was greatest in forage sorghum inoculated with LBLL, intermediate in sorghum sudan inoculated with LBLL, and gradually increased in both hybrids with storage length from 15 to 90 d ($P < 0.01$). Silage aerobic stability was 126 h greater, on average, for LBLL compared with CON and LPPP ($P < 0.01$). Ruminal in situ starch disappearance was increased from d 15 to 90 (54.5 vs. 59.3% of total starch, respectively; $P < 0.04$). Overall, microbial inoculation with LBLL improved aerobic stability of both hybrids. Forage sorghum had lower lactic and total acids ($P < 0.01$), however, it had greater starch digestibility ($P = 0.01$) than sorghum sudan. Storage length linearly increased starch digestibility of both hybrids, which indicates that storage length may be a useful management tool to optimize starch utilization.

Key Words: aerobic stability, starch disappearance, sorghum silage

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T56 Neutrophil immune dysfunction induced by acetoacetic acid via TLR2/4-NF-κB signaling pathway in dairy cows. H. Ding*[1], Y. Li[1], S. Feng[1], J. Li[1], X. Wang[1], J. Wu[1], Y. Liang[2], and J. J. Loor[2], [1]*Anhui Agricultural University*, *Hefei, Anhui, China*, [2]*University of Illinois at Urbana-Champaign*, *Urbana, IL*.

The objective of the current study was to investigate the effects of acetoacetic acid (ACAC) on mRNA and protein abundance related to the toll-like receptor 2/4-nuclear factor-κB (TLR2/4-NF-κB) signaling pathway in neutrophils (PMN) during the induction of inflammatory response in dairy cows. Nonketotic (n = 8) and ketotic (n = 8) Holstein cows were selected based on serum concentrations of β-hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), ACAC and glucose. PMN were treated with 0.6, 1.2 and 1.8 mM ACAC using 0 m*M* ACAC as control group, with or without ammonium pyrrolidinedithiocarbamate (PDTC, 10 μ*M*, 30 min), and N-acetylcysteine (NAC, 10 m*M*, 30 min). Total RNA and protein were used for quantitative real-time polymerase chain reaction and Western blotting, respectively. One-way ANOVA was performed by Statistical Package for the Social Science (SPSS) 17.0 and multiple comparisons of data were done using the Duncan test. Compared with nonketotic cows, serum concentrations of BHBA, NEFA, ACAC, hydrogen peroxide, malondialdehyde (MDA), glutathione peroxidase and tumor necrosis factor α (TNF-α) increased ($P < 0.05$) while glucose and catalase decreased ($P < 0.01$). Compared with control group, IKKβ activity, the protein abundance of TLR2 and phosphorylated-NF-κB p65 (p-NF-κB p65) in PMN increased in response to ACAC ($P < 0.01$). Additionally, ACAC increased transcription activity of p65. Moreover, ACAC treatment led to greater protein abundance of TNF-α ($P < 0.01$), while decrease after NAC treatment. ACAC could increase MDA ($P < 0.01$), where decrease the concentrations of CAT and Cu/Zn SOD ($P < 0.05$) in PMN. However, abundance of p-NF-κB p65, transcription activity of p65 and target TNF-α were decreased in PDTC + ACAC group compared with ACAC (1.2 mM) group. In conclusion, inflammation and oxidative stress existed in the ketotic dairy cows and ACAC caused inflammatory damage and immune dysfunction in PMN by activating the TLR2/4-NF-κB inflammatory signaling transduction pathway, moreover, which could be alleviated by NAC.

Key Words: dairy cow, immune dysfunction, TLR2/4-NF-κB

T57 Effects of LPS administration and subsequent nutrient restriction on systemic inflammation and hepatic steatosis in lactating dairy cows. E. A. Horst*, S. Rodriguez-Jimenez, E. J. Mayorga, M. A. Abeyta, B. M. Goetz, S. Carta, and L. H. Baumgard, *Iowa State University*, *Ames, IA*.

Objectives were to evaluate effects of repeated LPS exposure followed by nutrient restriction on inflammation and fatty liver in lactating cows. Cows (631 ± 16 kg BW; 124 ± 15 DIM) were enrolled in a study consisting of 3 experimental periods (P); during P1 (5d) cows were ad libitum-fed and baseline data were obtained. During P2 (7d), cows were assigned to 1 of 2 treatments: 1) saline-infused and pair-fed (PFFR; 5 mL saline on d1, 3, and 5; n = 6) or 2) LPS-infused and ad libitum-fed (LPSFR; 0.2, 0.8, and 1.6 μg LPS/kg BW on d1, 3, and 5, respectively; n = 6). During P3 (7d), all cows were feed restricted to 50% of P1 feed intake. On d3 of P3, LPSFR cows received an LPS bolus (0.8 μg LPS/kg BW) whereas PFFR cows received saline. Blood samples were collected on d1 and 3 of P1 and d1, 3, 5, and 7 of P2 and P3. Liver biopsies were obtained on d3 of P1, d7 of P2, and d3 and 7 of P3. Data were analyzed using PROC MIXED. Mild hyperthermia was observed for ~6h following each LPS bolus on d1, 3, and 5 of P2 and on d3 of P3 (0.6, 0.6, 0.8, and 0.5°C, respectively; $P \leq 0.04$). Circulating LBP, SAA, and Hp increased in LPSFR relative to PFFR during P2 (3-, 4-, and 7-fold, respectively; $P \leq 0.04$); LBP and SAA remained increased (2-fold) in LPSFR during P3 ($P < 0.01$). Circulating Hp from LPSFR steadily increased for the first 5d of P3 (5-fold relative to PFFR) and then returned to baseline ($P < 0.01$). Serum albumin and cholesterol from LPSFR decreased sharply from d1–3 of P2 and remained decreased for the duration of P2 relative to PFFR (12 and 16%, respectively; $P \leq 0.06$). During P3, albumin and cholesterol decreased in LPSFR vs. PFFR cows (10 and 20%, respectively; $P = 0.01$). Liver fat content did not differ between treatments at the end of P2. Liver fat from LPSFR increased with time during P3, whereas it remained similar in PFFR cows ($P = 0.04$). Relative to P1, liver fat from both treatments increased during P3 (49 and 76% in LPSFR and PFFR cows, respectively; $P < 0.01$). In summary, repeated LPS administration followed by nutrient restriction induced a sustained systemic inflammatory response and increased liver fat content.

Key Words: inflammation, feed restriction

T58 Mechanistic target of rapamycin pathway components and proteins associated with amino acid metabolism differ among adipose depots and mammary gland in late-lactation Holstein cows. Y. Liang*[1], N. Ma[1,2], D. N. Coleman[1], F. Liu[1,3], Y. Li[1,4], H. Y. Ding[1,4], F. F. Cardoso[1], F. C. Cardoso[1], and J. J. Loor[1], [1]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana*, *Urbana, IL*, [2]*College of Veterinary Medicine, Nanjing Agricultural University*, *Nanjing, China*, [3]*Department of Animal Science and Veterinary Medicine, Henan Agricultural University*, *Zhengzhou, China*, [4]*Department of Veterinary Medicine, College of Animal Science and Technology, Anhui Agricultural University*, *Hefei, China*.

Branched-chain amino acids (BCAA), leucine in particular, are well-known regulators of the mechanistic target of rapamycin (mTOR), hence, they exert control on protein synthesis and cell growth. Mammary gland is a major site for protein synthesis during lactation, while adipose depots (AT) might be major sites for BCAA uptake and initial catabolism. The objective of this study was to characterize the protein profiles of mTOR pathway components, AA transporters, and BCAA catabolism among mammary gland, omental, mesenteric and s.c. AT. Four Holstein cows (parity 4 ± 1.4, DIM 248 ± 38 before slaughter; mean ± SD) were used in this study. All cows were fed a common lactation diet before slaughter. Subcutaneous, omental, and mesenteric AT along with mammary gland tissue were collected immediately after slaughter. Statistical analysis was performed using the MIXED model in SAS 9.4 with tissue as fixed effect. Adipose tissue had greater phosphorylated (p) ratio of protein kinase B (AKT) to total AKT than mammary gland ($P < 0.05$). Mesenteric and SAT had greater activation of AKT (p-AKT/total AKT) than omental AT ($P < 0.05$) suggesting the later might be less insulin-sensitive (at least in late-lactation). Omental and SAT had greater activation of mTOR (p-mTOR/total mTOR) compared with mammary tissue ($P < 0.05$). However, compared with mesenteric AT, mammary tissue had greater activation of mTOR ($P < 0.05$). Branched-chain ketoacid dehydrogenase kinase (BCKDK) is a key enzyme in regulating BCAA catabolism. Glutathione S-transferase Mu 1 (GSTM1) modulates glutathione synthesis. Compared with mammary gland, AT depots had greater protein abundance of BCKDK and GSTM1 ($P < 0.05$), suggesting that AT depots might play an important role in regulating BCAA catabolism and oxidative stress. Solute carrier family 1 member 5 (SLC1A5) was only detectable in SAT and omental AT. Overall, data suggest that adipose depots play a role in BCAA metabolism and have antioxidant mechanisms that might help preserve tissue function such as insulin sensitivity.

Key Words: adipose depots, branched-chain amino acid, mechanistic target of rapamycin

T59 Impacts of endocrine signals altered by heat stress on mammary gland gene expression during the dry period. V. Ouellet*, V. Lantigua, A. L. Skibiel, T. F. Fabris, J. Laporta, and G. E. Dahl, *De-*

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Late gestation heat stress increases blood prolactin (PRL) and decreases estrogen (E) concentrations in cows. These alterations may disturb mammary gland remodeling, thereby resulting in a reduction in subsequent milk yield. We investigated the effects of altered endocrine environment induced by heat stress on mammary gland gene expression at different stages of the dry period. Mammary gland biopsies of cows that were either cooled (CL, n = 7) or heat stressed (HT, n = 7) during the dry period were collected at d3 and 35 relative to dry off and equally divided in 3 explants. Tissue explants were then incubated in vitro for 24h in 1 of 3 mediums: 1) Basal (Bm): no PRL or E; 2) CL-mimic (Cm): Bm + 20ng/mL PRL + 5.8ng/mL E or 3) HT-mimic (Hm): Bm + 40 ng/mL PRL + 2.9ng/mL E. Real Time qPCR BioMark Dynamic Array Integrated Fluidic Circuits was used to assess the expression of 66 genes encompassing multiple pathways (e.g., apoptosis, autophagy, proliferation, immunity). Statistical significance between in vivo (CL, HT) and in vitro (Bm, Cm, Hm) treatments and their interaction were determined by ANOVA and assessed separately by time period. In vivo treatment impacted genes related to apoptosis (*CASP3,9*), cell proliferation (*EGF*, *MAPK3*, *PDK1*, *PIK3R2*), and autophagy (*BECN1*), which were downregulated in explants from HT cows relative to CL at d3, while immunity genes (*TGFBP3, NFKB1*) were upregulated. Upregulation of genes related to proliferation (*CCND1*), and autophagy (*MAP1LC3A*) was observed in explants from HT relative to CL cows at d35. Expression of proinflammatory cytokines (*CXCL2*, *TNF, IL1A, IL1B, and IL6)* increased in explants incubated in Cm and Hm relative to Bm at d35. Pro-apoptotic gene *CASP8* and immune-related gene *LBP* were downregulated at d35 in explants from CL cows incubated in Cm relative to Bm whereas they were upregulated in explants from HT cows incubated in Cm relative to Bm. Collectively, our results indicate that heat stress influences the expression of genes involved in key cellular processes occurring during the dry period and that these modifications may be mediated by hormonal alterations.

Key Words: Fluidigm, heat stress, endocrine signal

T60 Comparison of ruminal and serum short-chain fatty acids concentrations in dairy cows with different levels of milk production.

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Short-chain fatty acids (SCFA) are the product of microbial fermentation in the rumen and are the main source of energy used by dairy cows for milk production. This pilot study aimed to compare the concentration of SCFA in ruminal fluids and serum collected from second lactation Holstein dairy cows with different levels of milk production. Rumen and serum samples were collected at 65 ± 8.5 DIM from 24 randomly selected cows. Cows were divided in 3 phenotypic groups based milk production: low producing (LP; n = 8) with 41.8 ± 1.2 kg/d yield, moderate producing (MP; n = 10) with 54.9 ± 1.1 kg/d yield, and high producing (HP; n = 6) with 61.2 ± 1.5 kg/d yield. Rumen samples were collected using non-invasive oro-ruminal sampling device (Flora Rumen Scoop, Profs-Product) and serum samples were collected by puncture of the coccygeal blood vessels using vacuum tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Rumen and serum samples stored at −80°C until metabolomics analysis. The concentrations of acetic, propionic, and butyric acids in rumen fluid and serum were determined using liquid chromatography-mass spectrometry. The differences of SCFA concentrations in rumen fluid and serum between the cohorts was analyzed using 2-tailed *t*-test with Bonferroni-Holm correction for multiple comparisons. The concentration of SCFA in rumen fluid did not differ between phenotypic groups; however, the concentration of propionic acid in serum was lower in the LP (135 ± 40 ug/g) cows when compared with that in the MP (235 ± 132 ug/g) and HP groups (185 ± 55 ug/g) (LP vs. MP, $P = 0.04$; Lp vs HP, $P = 0.09$). Our results suggest that the concentration of propionic acid in serum is associated with milk production more than the concentration of propionic acid in rumen fluid.

Key Words: short-chain fatty acids, milk production, rumen fluid

T61 Adipose and muscle transcriptome analysis of dairy calves supplemented with 5-hydroxytryptophan.

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Serotonin is a monoamine that regulates processes such as energy balance and immune function. Manipulating this pathway in growing dairy calves could promote growth and development by modulating functions and signaling pathways within key organs. We characterized the adipose and muscle transcriptome of pre-weaned calves with increased serotonin bioavailability through the elucidation of differentially expressed genes (DEGs) and biological pathways. Holstein bull calves (21 ± 2 d old) were fed milk replacer (8 L/d) supplemented with saline (CON, 8 mL/d n = 4) or 5-hydroxytryptophan (5-HTP, 90 mg/d, n = 4) for 10 d. Calves were euthanized on d 10 to harvest adipose and muscle tissue for RNA-Sequencing and histological analyses. RNA-Seq (NovaSeq 6000) data analysis included read quality control and processing (FastQC and Trim Galore), genome alignment (Hisat and ARS-UCD1.2), transcript reconstruction (Cufflinks and Cuffmerge) and read counting (htseq-count). Differentially expressed genes (FDR ≤ 0.10) were detected using the *R* package edgeR. The enrichment of Gene Ontology terms with DEGs was analyzed using Fisher's exact test. To quantify cell number and surface area, H&E staining of adipose and muscle was performed. 5-HTP-fed calves had greater number ($P = 0.001$) but smaller adipocytes ($P = 0.08$) and similar muscle fiber number relative to CON. Overall, there were 9 and 18 DEGs within adipose and muscle, respectively. Notably, interferon δ inducible protein 47 (*IFI47*) was upregulated in both tissues ($\log_2$ fold change = 6.2 and 6.8). Significant biological pathways ($P < 0.01$) related to cell processes, such as regulation of cell proliferation, apoptosis and differentiation were identified in muscle. Other significant pathways were related to inflammatory response, neutrophil chemotaxis and cytokine-mediated signaling. In adipose, pathways related to intracellular signaling of small GTPases, extracellular matrix remodeling and cell migration were identified ($P < 0.05$). Manipulating the serotonin pathway in pre-weaned calves through 5-HTP supplementation influences biological functions and signaling pathways which play essential roles in metabolic homeostasis and immune function.

Key Words: serotonin

T62 A fluorescence resonance energy transfer approach to determine intracellular bioavailability of zinc in blood samples using a bovine mammary epithelial cell model.

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This study evaluated the intracellular bioavailability of Zn in blood plasma and serum from dairy cows using a Zn fluorescent resonance energy transfer (FRET) system. Bovine mammary alveolar epithelial cells (MacT) were incubated with either serum or plasma at 5, 10, 25, 50, 75, and 100% diluted with reduced serum medium (OptiMEM). Before transfection, MacT cells were cultivated in high glucose Dulbecco modified Eagle's medium (DMEM) with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin and Fungizone Antimycotic. The plasmid used in this study was the peZinCh-NB (Addgene) designed to detect intracellular Zn through FRET technology. Cells were seeded 24 h before transfection at 30,000 cells/well in a 96-well plate. Cells were transfected with Lipofectamine 3000 at 0.3 uL/well and at 150 ng/well of plasmid in OptiMEM. Free intracellular Zn can be depleted by chelating agents such as TPEN (*N*,*N*,*N*',*N*'-Tetrakis (2-pyridylmethyl) ethylenediamine). Hence, additional treatments included 5 μM of TPEN(TPEN), 50μM of Zn (Zn), and 50μM of Zn+5 μM of TPEN (Zn+TPEN). An inverted fluorescent microscope for live imaging (EVOS FL Auto) equipped with an environment-controlled chamber at 37°C and 5.0% of CO_2 was used to take 4 pictures/well at 4x magnification at 0 and 24 h post-treatment. Quantification of FRET signal and cell viability were assessed using the CellProfiler software. Data were analyzed using the PROC MIXED of SAS. A treatment effect ($P < 0.01$) in FRET signal was observed in serum and a trend ($P = 0.11$) in plasma samples. There was an evident decline in FRET signal as serum % was increased with lower ($P < 0.01$) FRET at 100% serum, this suggests an increase in intracellular Zn. There were no differences in FRET across plasma %. The cell viability at 24h was similar across serum ($P \geq 0.54$) and plasma ($P \geq 0.34$) treatments. These preliminary data indicate that the available Zn in serum can be detected via FRET in bovine cells. To expand on these effects, gene expression and Zn concentration will be analyzed.

Key Words: zinc, cell culture, fluorescent protein

T63 Methionine and arginine supply alleviate oxidative stress and inflammation in subcutaneous bovine adipose explants challenged with H_2O_2. N. Ma*[1,2], Y. Liang[1], D. N. Coleman[1], F. Liu[1,3], Y. Li[1,4], H. Ding[1,4], F. F. Cardoso[1], F. C. Cardoso[1], X. Shen[2], and J. J. Loor[1], [1]*University of Illinois*, *Urbana, IL*, [2]*Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [3]*Henan Agricultural University*, *Zhengzhou, Henan, China*, [4]*Anhui Agricultural University*, *Hefei, Anhui, China*.

Methionine (Met) and arginine (Arg) alter protein synthesis through the mechanistic target of rapamycin (mTOR), and regulate oxidative stress (OS) partly through their metabolism to glutathione and polyamines. We examined if enhanced supply of Met or Arg in adipose tissue (AT) explants challenged with H_2O_2 altered OS. Subcutaneous AT was harvested from 4 Holstein cows (parity 4 ± 1.4, DIM 248 ± 38 before slaughter; mean ± SD). Tissue was incubated for 4 h with the following: control medium with an "ideal" profile of essential amino acids (AA) (IPAA, Lys:Met 2.9:1, Lys:Arg 2:1), IPAA plus 100 μM H_2O_2 (OS), H_2O_2 plus increased Met (OS+Met; Lys:Met 2.5:1), H_2O_2 plus increased Arg (OS+Arg; Lys:Arg 1:1), or H_2O_2 plus increased Met/Arg (OS+Met/Arg; Lys:Met 2.5:1 and Lys:Arg 1:1). The following results were protein expression measured by Western Blot. Preplanned orthogonal contrasts were IPAA vs. OS, OS vs. the average of OS+Met, OS+Arg, and OS+Met/Arg, OS+Met vs. OS+Met/Arg and OS+Arg vs. OS+Met/Arg. The MIXED procedure of SAS 9.4 was used (SAS Institute Inc., Cary, NC). Challenge with OS lowered ($P < 0.01$) ratio of phosphorylated (p)-mTOR to total mTOR, but greater supply of Met and Arg reversed this effect. Compared with Arg, supply of Met/Arg during OS challenge led to greater ($P < 0.01$) p-mTOR/mTOR. In spite of lower ($P < 0.01$) abundance of kelch-like ECH-associated protein 1 (KEAP1) with Met and Arg compared with OS, there was no effect ($P = 0.25$) of the AA on nuclear factor erythroid 2-related factor 2 (NRF2). Compared with Met or Arg alone, abundance of the proinflammatory transcription factor NF-κB p65 was lower when both AA were supplied together during OS challenge. Abundance of hydroxycarboxylic acid receptor 2 (HCAR2), known to elicit anti-inflammatory effects in non-ruminant AT, was lower with OS challenge, but supply of AA did not ($P = 0.19$) alleviate this effect. Overall, data suggest that enhanced supply of Met and Arg could alleviate adipose tissue oxidative stress and inflammation induced by OS. The relevance of these results in vivo merit further study.

Key Words: amino acid, oxidative stress, inflammation

T64 Effect of an ad libitum milk supply during the first three weeks of life of dairy calves on heart rate and heart rate variability during feeding. S. Wiedemann*[1], L. Prokop[2], G. Hoffmann[3], and M. Kaske[4], [1]*Rhine-Waal University of Applied Sciences, Livestock Sciences and Environmental Impacts*, *Kleve, Germany*, [2]*University of Kiel, Institute of Animal Breeding and Husbandry*, *Kiel, Germany*, [3]*Leibniz Institute for Agricultural Engineering and Bioeconomy, Department Engineering for Livestock Management*, *Potsdam, Germany*, [4]*University of Zurich, Vetsuisse Faculty, Department for Farm Animals*, *Zurich, Switzerland*.

The objective of this study was to investigate the effect of nutrient supply during early postnatal life on heart rate (HR) and heart rate variability (HRV) as indicators of the cardiac response. Male and female calves were fed either a restrictive milk allowance twice per day (6 L/d; RES; n = 28) or an unlimited amount of milk (ad libitum; ADL; n = 28) during the first 3 weeks of life. All calves were housed in individual straw bedded hutches. To study the cardiac response to the feeding process on d 21 of life HR, HRV and variables in the time- and frequency domain were measured continuously using a portable recording system. Six time windows of 5 min each were chosen: resting time at 05.00 a.m., start of personnel activity in the barn, 15 min before feeding, during feeding, 15 min after feeding and 1 h after feeding. During resting RES calves had a very low HR (95.6 ± 6.1 beats per min) indicating a reduced metabolic activity in comparison to ADL calves (120.4 ± 6.0 beats per min; $P < 0.05$). During feeding HR reached peak values which were comparable in both groups, however the increase in HR was higher in RES calves. HRV variables of the time- and frequency domain indicated a shift toward a sympathetic dominance in the balance of the autonomic nervous system during feeding time particularly in RES calves. Differences between resting and feeding values were demonstrated in RES calves at low frequency and high frequency power, whereas no differences were observed in ADL calves. It can be concluded that particularly the increase in the HR indicated higher excitement in RES calves during times of feeding.

Key Words: heart rate, heart rate variability, ad libitum feeding of calves

T65 Somatotropin increases plasma ceramide concentrations in relation to enhanced milk yield in Holstein dairy cows. A. N. Davis*[1], W. A. Myers[1], C. Chang[1], B. N. Tate[1], J. E. Rico[1], M. Moniruzzaman[2], N. J. Haughey[2], and J. W. McFadden[1], [1]*Cornell University*, *Ithaca, NY*, [2]*Johns Hopkins University*, *Baltimore, MD*.

Recombinant bovine somatotropin (rbST) changes metabolism to spare glucose for milk synthesis in cows. Ceramide inhibits insulin responsiveness in bovine adipocytes, and the accumulation of ceramide in circulation and tissues is associated with insulin resistance and milk production in cows. The mechanisms by which rbST supports lactation may involve ceramide. Eight multiparous lactating Holstein cows were enrolled in a 2 × 2 replicated Latin square design with 14-d periods. Cows received a single rbST injection (Posilac; Elanco Animal Health, Indianapolis, IN; 0.062 mg/kg BW) or no injection. An epinephrine challenge, insulin tolerance test, and liver biopsy were performed. Plasma glucose and total fatty acids (FA) were measured using colorimetry. Plasma insulin concentrations were determined using radioimmunoassay. Plasma and liver ceramides were quantified using mass spec. Data were analyzed using a mixed model. Somatotropin increased yields fat-corrected milk and energy-corrected milk, and feed efficiency (energy-corrected milk/dry matter intake; $P < 0.01$). All cows started in positive energy balance; however, rbST-treated cows entered negative energy balance by d 3 ($P < 0.05$; not observed for control cows). Area-under-the-curves for FA in response to epinephrine and insulin were greater in rbST-treated cows ($P < 0.05$). In response to insulin, glucose concentrations (20 and 30 min post-challenge) and insulin area under the curve were higher with rbST treatment ($P < 0.05$, 0.10, and 0.01, respectively). Somatotropin tended to reduce glucose clearance rate at 20 min following the insulin challenge ($P = 0.10$). Treating cows with rbST increased plasma total and very-long-chain (C22:0-, C24:0-, and C26:0-) ceramide concentrations ($P < 0.01$). Liver C18:0-ceramide concentrations decreased; however, no other liver ceramides were altered with rbST treatment. Plasma ceramides were positively correlated with circulating FA ($r = 0.57$; $P < 0.05$) and milk yield ($r = 0.63$; $P < 0.05$). We conclude that rbST administration increases plasma ceramide concentrations in association with increased milk production in Holstein cows.

Key Words: ceramide, dairy cow, somatotropin

T66 Associations of mild heat stress and genetic lineage with dry matter intake, water intake, rectal temperature, and active ghrelin concentration in Holstein heifers. L. Han*, C. Dechow, and A. Macrina, *Department of Dairy and Animal Science, The Pennsylvania State University, State College, PA.*

The active form of the hormone ghrelin (aGHR) is involved with the regulation of feed intake, thermogenesis and energy expenditure in Holstein heifers. The objectives of this experiment were to determine 1) how ambient temperature (AT) and genetic lineage (modern genetic, MG; 1950s era sire genetics, OG) affect dry matter intake (DMI), water intake (WI), rectal temperature (RT, pre-feeding and 7h post-feeding), aGHR (pre-feeding, 1.5h post-feeding, and 7h post-feeding), and 2) associations among aGHR, DMI, and RT. Eight pregnant heifers (4 MG and 4 OG) were assigned to 7d heat treatments in a 2 × 2 Latin-square design. Each treatment group contained 4 heifers (2 MG and 2 OG). All heifers were fed once daily at 9:00 a.m. The average daily AT was 28°C for heat treatment while the average daily AT for the control environment was 22°C. Temperature-humidity-index was also considered with results nearly identical to those for AT. A repeated measures analysis of DMI, WI, RT, and aGHR was used to determine effects of genetic lineage and the covariate AT as well as associations among aGHR, DMI, and RT. The repeated effect was date with the subject heifer and significance declared at $P < 0.05$. Body weight (514 ± 33 kg), wither height (1.47 ± 0.03 m), DMI (9.9 ± 0.9 kg), WI (35.3 ± 4.7 L), RT (38.6 ± 0.27°C), and aGHR (389 ± 177 pg/mL) did not differ between MG and OG; OG had higher body condition scores than MG (4.13 vs 3.13) which indicates that MG had lower body fat percentage. AT increased RT (slope = 0.025) and WI (slope = 0.42), decreased DMI (slope = −0.09), and had no impact on aGHR (slope = 3.72, $P = 0.22$). aGHR 7h post-feeding had a positive association with RT pre-feeding (slope = 0.0005) and 7h post feeding (slope = 0.0003, $P = 0.06$). Higher DMI was associated with lower pre-feeding (slope = −0.001) and 1.5h post feeding aGHR (slope = −0.002). These results indicate that aGHR is associated with DMI and RT regulation.

Key Words: ghrelin, temperature, DMI

T67 Methionine and arginine alter antioxidant and mechanistic target of rapamycin (mTOR) protein abundance in mammary explants stimulated with hydrogen peroxide. D. N. Coleman*[1], P. Totakul[1,2], N. Onjea-uea[1,3], Y. Liang[1], F. F. Cardoso[1], F. C. Cardoso[1], and J. J. Loor[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand,* [3]*Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand.*

The objective was to investigate effects of enhanced Met and Arg supply alone or in combination on protein abundance of mechanistic target of rapamycin (mTOR) and antioxidant signaling pathways in mammary gland explants during stimulation with hydrogen peroxide (HP). Mammary tissue from 4 Holstein cows (parity 4 ± 1.4, DIM 248 ± 38 before slaughter; mean ± SD) was incubated in duplicate for 4, 8, 12 or 24h with 1 of the following media: ideal profile of essential AA as the control (IPAA; Lys:Met 2.9:1, Lys:Arg 2:1), increased Met (incMet; Lys:Met 2.5:1), increased Arg (incArg; Lys:Arg 1:1), or incMet plus incArg (Lys:Met 2.5:1 Lys:Arg 1:1) with or without exogenous HP (600 µM). Total protein was extracted to measure protein abundance using Western blot analysis. Data were analyzed as a 2 × 2 × 2 factorial using the MIXED procedure of SAS 9.4. Data from the IPAA treatment were analyzed over time to investigate the effect of incubation time. No differences were detected in the abundance of glutathione peroxidase 1 (GPX1), mTOR, phosphorylated (p) mTOR, AKT or p-AKT ($P > 0.10$) over time in IPAA treatments, indicating that mammary explants may be cultured for up to 24 h. After 8 h of incubation a tendency for an interaction between Met and HP was observed for GPX1 at 8 h ($P = 0.09$); stimulation with HP led to greater abundance of GPX1 compared with incubation without hydrogen peroxide, Met or Met and HP. However, there was no difference in the abundance of GPX1 between tissue treated with Met alone or in combination with HP. The abundance of p-mTOR was also greater in explants incubated with HP ($P = 0.01$). This increase in p-mTOR was associated with a tendency for an increase in the ratio of p-mTOR:total mTOR in explants incubated with HP ($P = 0.09$). The protein abundance of mTOR also tended to be increased by Arg ($P = 0.09$). Overall, preliminary evaluation of proteins indicates that supplying Met to mammary explants may help to maintain homeostasis in antioxidant metabolism during stimulation with HP. Further, supplying Arg to mammary explants may lead to increased mTOR signaling.

Key Words: amino acids, oxidative stress

T68 Effects of an immunomodulatory feed additive on biomarkers of inflammation and oxylipid profile in blood of transition cows. C. S. Takiya*[1], L. K. Mamedova[1,2], L. Sordillo[2], J. Gandy[2], M. Garcia[3], E. E. Gultepe[4], D. E. Nuzback[3], and B. J. Bradford[1,2], [1]*Kansas State University, Manhattan, KS,* [2]*Michigan State University, East Lansing, MI,* [3]*Phibro Animal Health, Teaneck, NJ,* [4]*Afyon Kocatepe University, Afyonkarahisar, Turkey.*

Dairy cows experience an inflammatory state during the postpartum period even in the absence of disease. However, cows are prone to developing metabolic diseases when inflammation is exacerbated. Oxylipids are signaling molecules derived from oxidation of long-chain fatty acids that exert complex control over inflammation. OmniGen-AF (OMN, Phibro Animal Health, Teaneck, NJ) is a commercial feed additive that has shown positive impacts on immunity and performance of dairy cows. Twenty-eight multiparous cows were blocked by expected calving date and assigned to control or OMN (56 g/d top-dressed) from the day of dry-off (−67 ± 3.0 d relative to actual calving date) until 49 d in milk. Blood samples were collected at enrollment, d −30, −14, −7, 1, 14, 28, and 42 relative to parturition (RTP) for assessment of plasma haptoglobin (Hp) and α-1-acid glycoprotein (AGP) concentrations; samples from d −14, 1, 7, 14 RTP were analyzed for oxylipid profiles. Haptoglobin and AGP were analyzed based on peroxidase activity and by ELISA (ICL Inc., Portland, OR), respectively. Plasma oxylipids were analyzed by LC/MS/MS. Rectal temperature was measured daily after parturition. Data were submitted to ANOVA with fixed effect of treatment, time, their interaction, and random effects of cow and block; fixed effects of parity group, temperature and humidity-index, and all interactions were also tested. Significance was declared at $P < 0.05$. Cows fed OMN had similar ($P = 0.11$) rectal temperature as CON (38.67 vs. 38.72 ± 0.033°C, respectively). No treatment nor treatment × time effects were detected for Hp or AGP. Among 33 oxylipids detected, OMN increased ($P = 0.018$) plasma 6-keto prostaglandin-1α concentration, which is a stable metabolite of prostacyclin I_2. Treatment × time interactions ($P \leq 0.048$) were observed for thromboxane B_2 and 12-hydroxyheptadecatrienoic acid (derived from arachidonic acid via cyclooxygenase), where OMN increased blood concentrations of these metabolites on d −14 RTP. This study demonstrated that OMN can alter pro-inflammatory and resolving class oxylipids.

Key Words: eicosanoid, immunity, rectal temperature

T69 Effects of an immunomodulatory feed additive on granulocyte activity in peripheral blood and uterus of early lactating cows. C. S. Takiya*[1], J. L. McGill[2], L. K. Mamedova[1,3], A. L. A. Scanavez[1], R. Rusk[1], L. G. D. Mendonça[1], M. Garcia[4], D. E. Nuzback[4], and B. J. Bradford[1,3], [1]*Kansas State University, Manhattan, KS,* [2]*Iowa State University, Ames, IA,* [3]*Michigan State University, East Lansing, MI,* [4]*Phibro Animal Health, Teaneck, NJ.*

Parturition is a stressful event that causes endometrial damage and facilitates uterine infections. OmniGen-AF (OMN, Phibro Animal Health, Teaneck, NJ) is a feed additive that has improved measurements of cellular immunity and postpartum uterine health of mammals. However, OMN effects on immune cells isolated from endometrium have not been explored. Twenty-eight healthy multiparous cows were blocked by expected calving date and assigned to control or OMN (56 g/d top-dressed) from dry-off (67 ± 3.0 d before calving date) until 49 d in milk. Endometrial samples were collected on d 12 ± 2, 21 ± 3, and 38 ± 3 postpartum using the cytobrush technique. Blood samples were also collected after cytobrush sampling. Blood and uterine brush samples were prepared for flow cytometry to measure oxidative burst of granulocytes after phorbol myristate acetate stimulation. Labeled antibodies (CH138a and CD45) and dihydrorhodamine were used to identify granulocytes and quantify oxidative burst, respectively. Granulocyte response (GR) was calculated as stimulated – basal oxidative burst. Data were analyzed using the Mixed procedures of SAS to model the fixed effects of treatment, time, their interaction, and random effects of cow and block; fixed effects of parity group, temperature-humidity index, and all interactions were also tested in a stepwise backward elimination when $P > 0.15$. Significance was declared at $P < 0.05$. Granulocyte percentage in uterine samples did not differ between treatment groups (10.7 vs. 9.30 ± 2.8% for OMN and control, respectively) but decreased over lactation from 14.4 ± 3.7% on d 10 to 2.39 ± 4.61% on d 38. Similar GR, both in blood and uterine samples, was observed across treatment groups; however, a treatment × time interaction was observed in uterine GR. Cows fed OMN had lesser uterine GR on d 10 and greater uterine GR on d 38 compared with controls. Furthermore, OMN increased GR in parity 3+ cows but did not influence parity 2 cows. This study demonstrated that OMN did not alter neutrophil infiltration but modulated uterine GR in early lactation, with a greater impact on cows with 3 or more parities.

Key Words: OmniGen-AF, neutrophil, oxidative burst

T70 The effects of zinc chelator TPEN in bovine mammary epithelial cells evaluated via a fluorescence resonance energy transfer approach. R. Mohan* and J. S. Osorio, *Dairy and Food Science Department, South Dakota State University*, *Brookings, SD*.

Zinc plays important roles in many cellular functions. The free intracellular Zn is sensitive to chelating agents like TPEN (*N*,*N*,*N*',*N*'-Tetrakis (2-pyridylmethyl) ethylenediamine). Therefore, we evaluated the effect of TPEN on changes in the intracellular Zn in bovine mammary epithelial alveolar cells (MacT). Before transfection, MacT cells were cultivated in high glucose Dulbecco modified Eagle's medium (DMEM) with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin, and Fungizone Antimycotic. The plasmid used in this study was the peZinCh-NB (Addgene) designed to detect intracellular Zn through a fluorescence resonance energy transfer (FRET) technology. Cells were seeded 24h before transfection at 30,000 cells/well in a 96-well plate. Cells were transfected with Lipofectamine 3000 at 0.3 µL/well and 150 ng/well of plasmid in reduced serum medium (OptiMEM). The transfected cells were treated in triplicates with 5 µ*M* of TPEN (TPEN), 50 µ*M* of Zn (Zn), and 50 µ*M* of Zn + 5 µ*M* of TPEN (Zn+TPEN). An inverted fluorescent microscope for live imagining (EVOS FL Auto) equipped with an environment-controlled chamber at 37°C and 5.0% of CO_2 was used to take 4 pictures/well at 4x magnification at 0 and 24 h post-treatment. Quantification of fluorescence resonance energy transfer (FRET) signal and cell viability were assessed using the CellProfiler software. Data were analyzed using the PROC MIXED of SAS. A treatment × time effect ($P < 0.01$) was observed in FRET signal, where FRET was similar ($P \geq 0.18$) across treatments at 0h but resulted in greater ($P < 0.01$) FRET in TPEN in comparison to Zn and Zn+TPEN at 24h. This suggests lower intracellular Zn concentration in TPEN than Zn and Zn+TPEN at 24h. The cell viability at 24h was similar ($P \geq 0.68$) across treatments. These preliminary data indicate that TPEN is a viable model to study molecular alterations in Zn-depleted bovine cells and allow to evaluate additional conditions such as inflammation. FRET technology was sensitive to TPEN effects on intracellular Zn. To expand on these effects, gene expression and Zn concentration will be analyzed.

Key Words: zinc, cell culture, fluorescent protein

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T71 Effect of dry period length on risk of culling in the subsequent lactation. P. Pattamanont[1], M. I. Marcondes[2], and A. De Vries*[1], [1]*University of Florida*, *Gainesville, FL*, [2]*Federal University of Vicosa*, *Vicosa, MG, Brazil*.

The objective of this study was to quantify the effect of dry period length (days dry, DD) in the current lactation on the risk of culling in the subsequent lactation. For this retrospective study, we obtained DHI test records of 1,287,659 Holstein cows from 5,155 herds with the last dry date in 2014 or 2015 from DRMS, NC, USA. Three groups of adjacent lactations were constructed: 1 and 2 (par12; n = 605,082), 2 and 3 (par23, n = 362,169), and 3 to 6 (par36, n = 320,408). Included cows had between 21 to 120 DD which were classified into 9 categories (shortest 21–30 DD to longest 101–120 DD). Survival analysis was conducted with proc GLIMMIX in SAS with a 30-d piecewise exponential model with 9 DD categories, 2 time-dependent variables (4 seasons (winter, spring, summer, fall) and 4 reproductive statuses (open, pregnant, bred ≤ 30 d, bred > 30 d), and 5 covariates including total milk yield in the current lactation, milk yield on d 14 before dry-off, and DIM at dry-off. Next calvings, lactations in progress or cows sold for dairy purposes or reason-not-reported were censored. The model was run separately for each group for 0 to 60 and 61 to 450 DIM after calving to quantify the effects of dry period length on culling in early and later lactation. Hazard ratios (HR) for each DD category were calculated with the HR of the 51–60 DD category set to 1. The HR for the 0–60 DIM ranged from 0.81 (21–30 DD) to 1.73 (101–120 DD) for par12, 0.77 (21–30 DD) to 1.64 (101–120 DD) for par23, and 0.74 (21–30 DD) to 1.52 (101–120 DD) for par36. The HR for the 60–450 DIM were also generally greatest for long DD and smallest for short DD, but were closer to 1, implying that differences in DD had greatest effects early in lactation. Models without time-dependent variables and covariates showed greater HR for short DD (closer to 1) and greater HR for long DD (>2) compared with models that included these variables. In conclusion, a longer dry period length in the current lactation increased risk of culling in the subsequent lactation and needs to be considered when optimizing dry period lengths for individual cows.

Key Words: dry period, culling, survival

T72 Evaluation of research needs and management practices on organic, grass-fed dairy farms in the United States. M. A. Snider*[1], S. E. Ziegler[2], H. M. Darby[2], K. J. Soder[3], A. F. Brito[4], B. Beidler[5], S. Flack[6], S. L. Greenwood[1], and M. T. Niles[1], [1]*University of Vermont*, *Burlington, VT*, [2]*University of Vermont Extension*, *St. Albans, VT*, [3]*USDA-ARS, Pasture Systems and Watershed Management Research Unit*, *University Park, PA*, [4]*University of New Hampshire*, *Durham, NH*, [5]*Beidler Family Farm*, *Randolph, VT*, [6]*Sarah Flack Consulting*, *Enosburg Falls, VT*.

In the past decade, there has been an increase in organic dairy production, but producers are currently facing challenges similar to that of their conventional counterparts. Due to economic pressures and philosophical beliefs, many organic producers are transitioning to “grass-fed” production systems. Previous research has identified demographic, management, education, and research needs of organic dairy farms in the northeastern United States. However, there is a paucity of research assessing specific parameters relevant to grass-fed dairies. Therefore, the objectives of this study were to 1) assess demographics, perceptions, and production practices on US organic grass-fed dairy farms and 2) identify areas of research that may educate producers, as well as advance the organic grass-fed dairy industry. Grass-fed organic dairy production is defined as farms relying solely on forage sources, excluding corn silage, to meet nutritional needs. Data were analyzed using Stata 16.0 with Pearson correlations coefficients. Of the 351 questionnaires mailed, 164 producers responded. The majority of producers were located in NY, OH, WI, PA, and VT, with an average age of 47.6 years old, which is 10 years younger than the national average. Sixty percent of respondents reported that they were part of the plain community; 96.3% of surveyed farms were found to have been certified organic for an average of 10.3 years and 84.2% were grass-fed for 5.1 years. Farms averaged 219 acres of pasture with a herd size of 49 cows; however, 63.0% of farms reported needing to purchase additional forages. Producers (60.0%) indicated that the largest limitations to their success were forage quality and low milk production. Despite limitations, 71.8% of producers were satisfied with their milk production, correlating with a high level of understanding of forages and forage quality tests. Based on results, identified research needs include creating financial benchmarks, modifying forms of farmer communication, and assessing animal performance on pasture-based diets.

Key Words: grass-fed dairy, farmer perceptions, organic

T73 Effects of herd fertility on the economics of sexed semen in a high-producing, pasture-based dairy production system. D. Walsh*[1], A. Fahey[1], F. Mulligan[2], and M. Wallace[1], [1]*School of Agriculture and Food Science, University College Dublin*, *Dublin, Ireland*, [2]*School of Veterinary Medicine, University College Dublin*, *Dublin, Ireland*.

Sexed semen has the potential to increase genetic gain of dairy populations through increased selection intensity on the dam side. However, the use of sexed semen in the dairy industry has been limited by sexing accuracy, low conception rates, and cost. A stochastic simulation model was used to estimate the potential economic benefit of using sexed semen in heifers and heifers and lactating cows in a high producing, pasture-based system under 3 fertility scenarios. Three breeding strategies were modeled: (1) only heifers inseminated with sexed semen and cows inseminated with conventional unsexed semen (SSH); (2) both heifers and cows inseminated with sexed semen (SSHC); and (3) a reference scenario in which all heifers and cow were inseminated with conventional, unsexed semen (CONV). Each scenario was evaluated under 3 herd fertility states: ‘high’ (HF), ‘medium’ (MF) and ‘low’ (LF) which under the reference scenario corresponded to herd replacement rates of 21%, 25% and 31%, respectively. The model estimated the economic profit including the net present value of the genetic gain from increased selection intensity. The economic return from adoption of sexed semen strategies declined with reduced levels of baseline herd fertility turning negative in the LF state. The Sexed Semen Advantage (SSA) for HF-SSH, MF-SSH and LF-SSH scenarios were €30.61/cow ± 8.98, €27.45/cow ± 7.19 and €14.69/cow ± 11.06 respectively. However, the SSA for HF-SSHC, MF-SSHC and LF-SSHC scenarios were €49.14/cow ± 15.43, €18.46/cow ± 30.08, and €-19.30/cow ± 57.11. The range in economic profit for SSA for SSH was most sensitive to calf prices in HF-SSH and the pregnancy rate of sexed semen as a percentage of conventional in MF-SSH and LF-SSH. The range in economic profit for SSA for SSHC scenarios was most sensitive to sexed semen as a percentage of conventional in HF-SSHC, MF-SSHC and LF-SSHC. This study highlights the effect of baseline herd fertility state on the financial advantage of adopting sexed semen in a pasture-based, dairy production system.

Key Words: sexed semen, stochastic model, grazing

T74 Potentials and flaws of using 3-dimensional models to describe changes in energy reserves in dairy cattle. J. H. M. Viana[1], E. K. N. Arashiro[2], L. G. B. Siqueira[3], and L. G. R. Pereira*[3], [1]*Embrapa Recursos Genéticos e Biotecnologia - Embrapa*, *Brasília, DF, Brazil*, [2]*Universidade Federal Fluminense*, *Niterói, RJ, Brazil*, [3]*Embrapa Gado de Leite*, *Juiz de Fora, MG, Brazil*.

The mobilization of energy reserves during lactation determines changes in body shape, which are used to score body condition (BCS) by visual inspection. Recently, new strategies have been proposed to automatize BCS evaluation. Three-dimensional (3D) scanning can be used to obtain geometric data from body surface, however, its use to predict energy reserves still needs validation in cattle. In this study, structured infrared light scanning (Kinect sensor, Microsoft) was used to create 3D models of the rump and dorsal regions of Holstein x Gir crossbred dairy cows ($n = 65$). The 3D models were used to measure the average (right and left sides) area (A3D; mm^2) between the sky surface and a reference line drawn: 1) between transverse and spinous processes of the lumbar vertebra near the 13th rib, for the loin; 2) between *tuber coxae* and *tuber ischiae* prominences, for in the ilium-ischium depression; and 3) between both *tuber ischiae* prominences, for the ischiorectal fossa. Measurements were performed with a 3D modeling software (Rhinoceros 3D 5.0, McNeel). The subcutaneous fat thickness (SFT) was measured by ultrasonography, using a 3.5 MHz, liner array probe (Aquila Vet, Esaote), at the insertion of *biceps femoris* muscle and at the region of *longissimus dorsi* muscle, between 12th and 13th ribs. Cows were visually scored for BCS in a 1 to 5 scale. The association among A3D, SFT, and BCS was evaluated by correlation and regression analysis, correlations were compared using the Pearson and Filon's test (comparingcorrelations.org). All estimations of energy reserves by SFT and A3D were correlated with BSC ($P < 0.05$). However, A3D was equivalent to SCF for describing changes in BCS when measured at the rump ($R = 0.72$ vs. $R = 0.81$; $P > 0.05$), but not at the loin ($R = 0.47$ vs. $R = 0.81$; $P < 0.001$). There was a quadratic effect of BCS on A3D, and in cows with BCS above 4, SCF was better than A3D for describing variations in energy reserves ($P < 0.05$). In summary, changes in rump 3D surface geometry can be effectively used to estimate energy reserves in non-obese cows.

Key Words: 3-dimensional imaging, BCS, precision dairy farming

T75 Integrated solutions to maintain dairy production resilience under six different future scenarios in a regional food, energy, and water systems analysis. M. E. de Haro Martí*[1], J. Wilson[1], S. Elmer[2], P. Williams[3], and A. Kliskey[3], [1]*University of Idaho*, *Gooding, ID*, [2]*University of Idaho*, *Twin Falls, ID*, [3]*University of Idaho*, *Moscow, ID*.

Using the Magic Valley (MV), Idaho, as our case study of the integration of food, energy, and water systems (FEWS), we co-developed future scenarios and over 90 integrated solutions with local stakeholders. Agriculture and dairy production and processing are primary economic drivers in this system. Water from the Upper Snake River Basin supplies irrigated crops, cities, dairies and processors. All agriculture in the MV is irrigated. Idaho is the fourth largest dairy producing state in the USA and 73% of the dairy cows in Idaho are in the MV. We tested the resilience of the MV under 6 different scenarios of changing climate, demographics, adoption of resource recovery technologies, adoption of BMPs, and socio-economic development. Stakeholders and researchers explored reactions of the system to each scenario, and plausible integrated solutions to maintain the resilience of the FEWS. Watershed dynamics were modeled, as well as the impact of the different drivers on water availability, quality, and its impact on the rest of the FEWS. We describe 6 scenarios and the top 5 solutions per scenario that are pertinent to the dairy industry and could allow it to maintain resilience. Some of the scenarios would greatly impact dairies, including a mega drought, or drastic changes in demographics and economic activity. Under other scenarios the dairy industry thrives. In general, dairy can maintain a good level of resilience within the FEWS but may need to implement drastic solutions to be able to maintain its operations and economic viability, reduce environmental impacts, and adapt to new realities depending on the scenario. Solutions include changing feed crops, adopting several operation efficiencies like changes in breed selection or cow longevity and heifer replacement variations, type of dairy facilities, and implementation of BMPs and technologies for manure and nutrient management.

Key Words: food, energy, and water systems (FEWS), dairy resilience, sustainability

T76 Implementation of animal and herd phosphorus balance in the Ruminant Farm Systems (RuFaS) model. T. L. Hansen*[1], M. A. Sotirova[1], J. M. Tricarico[2], and K. F. Reed[1], [1]*Cornell University*, *Ithaca, NY*, [2]*Dairy Management Inc*, *Rosemont, IL*.

Dairy cattle require P for growth, energy metabolism, and acid-base balance, but excess P in agricultural systems is related to P runoff and eutrophication. Tools to assess nutrient management decisions and environmental implications are often static and evaluated at single time points; thus, the use of a dynamic model would improve decision making and evaluation of P nutrient flow. The Ruminant Farm Systems (RuFaS) model is a modern, modular approach to dairy system simulations incorporating nutrient flows through soil, crop, feed storage, animal, and manure management modules. The objective of this study was to build a dynamic P tracking module that maintains P mass balance at the animal, herd, and barn level. Initial P values of animals are calculated from P gestation requirements for simulated calves or as 0.72% BW for purchased cattle. Two strategies to update daily animal P values were tested: (1) estimate fecal P as the difference of intake and absorbed P or (2) estimate retained P as the difference between intake, secretion and excretion. For the first approach, NRC requirements were used to calculate absorbed P, and the constraint of P mass balance was established at the animal level. In a simulation of 125 lactating dairy cows with P intakes from 25 to 179 g/d, fecal P excretion ranged from −3.3 to 150 g/d. Negative fecal P excretion occurred at very low P intakes (≤0.2% DMI). Therefore, a second approach to calculate fecal P excretion and retained P in the animal module was developed using empirical equations to estimate fecal P excretion from P intake. Retained P ranged from −18.2 to 39.6 g/d and was highly correlated with P intake ($P < 0.001$, $r^2 = 0.979$). Animal P values are updated daily to account for individual animal P retention and excretion. Therefore, the second approach is more biologically relevant, accounts for BW changes, and maintains P mass balance at a herd level. Modeling the utilization and flow of P in addition to other nutrients within the animal module is critical to meet RuFaS objectives to identify and improve whole-farm production efficiency and minimize environmental impacts from dairy farms.

Key Words: cattle, mass balance, phosphorus

T77 An integrated sensor network for monitoring pastured cattle health and location. B. R. dos Reis* and R. R. White, *Virginia Tech*, *Blacksburg, VA*.

Although systems exist for monitoring health and location of cattle housed in confinement operations, analogous monitoring systems for pastured cattle are not commonly available. Our objective was to demonstrate an integrated sensor network for monitoring pastured cattle health and location. The integrated sensor network (Mahindra & Mahindra; Mumbai, India) links data obtained from a subcutaneous temperature sensor (Livestock Labs Inc., Pittsburgh, PA) with GPS data obtained from a solar-powered collar sensor (Sodaq; Hilversum, the Netherlands) via a satellite gateway (Hiber Global, Amsterdam, the Netherlands) to a cloud-based data storage platform. The subcutaneous temperature sensor is implanted surgically through a 5 mm vertical incision in the neck. After initiation, the sensor logs temperature and activity hourly and transmits data using a Bluetooth communication protocol to a solar-powered base station. If animals are out of range of the base station, the logger has capacity to store data for 2 weeks, or until animals come back into range of the station. Battery life on the sensors is expected to last 2 years. The base station is designed to use cellular communication but was retrofitted with LoRa communication for the purposes of this demonstration. The GPS collar sensors are commercially available and use solar power and LoRa-based communication. Data from the GPS collars and the implantable temperature sensors was transmitted via LoRa communication to the satellite gateway. The sensor network was deployed on 10 grazing cattle over a 6-mo period to test data reliability. The subcutaneous sensors were able to detect within-animal differences in body temperature associated with diurnal cycling, but improvements to base station design are needed to optimize data reliability (recover rates ~50%). Collars were able to reliably report on animal location for the duration of the trial. Further refinement of the data processing is needed to maximize the utility of this sensor network for monitoring cattle health and location.

Key Words: cattle health, GPS, precision technologies

T78 A time-series analysis of increasing milk productivity and yearly seasonality. M. Li*[1], V. E. Cabrera[1], and K. F. Reed[2], [1]*Department of Dairy Science, University of Wisconsin-Madison*, *Madison, WI*, [2]*Department of Animal Science, Cornell University*, *Ithaca, NY*.

US dairy cows are increasing their milk productivity due to improved genetics and farm management. Also, milk productivity shows seasonal patterns. It is critical to understand these trends and patterns for projecting production fluctuations. The objective of this study was to accurately forecast lactation performance to be used in a whole-farm simulation model, the Ruminant Farm System model (RuFaS). We quantified the seasonal effect and the trend of improvement on milk and milk component yields over the years. As input, we used a data set containing 10 million lactations records from 6 million Holstein's during the years 2006 to 2016. Each record included complete milk, fat, and protein yield standardized yearly. Although the data included 47 states, most records were from Wisconsin (26%), Pennsylvania (14%), and New York (13%). Lactation records were 40% 1st lactation, 28% 2nd lactation, and 32% later lactations. We decomposed a time series of the data with an additive model $y_t = S_t + T_t + R_t$, where y_t, S_t, T_t, and R_t were the yield, the seasonal, trend, and error terms, respectively, at time t. We found a strong seasonality effect and increasing trend for milk (MY), fat (FY), protein (PY), and energy-corrected milk (ECM) yields for all parities across the studied years (Table 1). The seasonality pattern indications (peak and trough period) and averages for the year 2016 and their change from 2006 are shown in the table. For forecasting yields in RuFaS, we used the Holt-Winters' seasonal forecasting method, which applies a triple exponential smoothing for milk production level, trend in milk production, and season, giving higher weight to the most recent data. The accuracy of forecasting was tested by identifying patterns from 2006 to 2015 and comparing our 2016 forecast with 2016 observed data. Observed values were in the 95% confidence interval of our predictions.

Table 1 (Abstr. T78).

Lactation	Trait	Peak	Trough	2016 (kg/cow per yr)	2016 − 2006 (kg/cow per yr)
1	ECM	Nov	Mar	10,956	908
	MY	Nov	Aug	10,537	728
	FY	Oct	Jan	390	35
	PY	Nov	Mar	322	28
2	ECM	Mar	Aug	12,524	1,145
	MY	Mar	Aug	12,142	993
	FY	Mar	Jul	442	43
	PY	Mar	Sep	370	35
Later	ECM	Jan	Jul	12,738	1,254
	MY	Jan	Jul	12,404	1,138
	FY	Dec	Jul	451	45
	PY	Dec	Jul	372	39

Key Words: RuFaS, calving season, time series

T79 Thermoelectric energy harvesting for wearable precision agriculture technologies. B. R. dos Reis*[1], B. Poudel[2], S. Priya[2], and R. R. White[1], [1]*Virginia Tech*, *Blacksburg, VA*, [2]*Penn State University*, *University Park, PA*.

Wearable precision agriculture devices are growing in popularity in dairy production systems globally. Currently, these devices rely on batteries with or without solar support as power sources. Although solar power works well for technologies deployed on pastured animals, equivalent alternative power sources for technologies deployed on animals in confinement systems are needed to improve the lifetime and usability of wearable technologies. Thermoelectric energy generators (TEG) are a promising technology that may be able to replace batteries in low-power wearable technologies. Our objective was to test the feasibility of using a TEG to power wearable precision agriculture technologies. The TEG harvests heat from the temperature differential between the animal's body and the ambient environment and converts that heat into available electrical energy that can be used to power microprocessors and associated sensors. To test the feasibility of leveraging the TEG to power microprocessor-based sensors, we constructed a prototype sensor consisting of a TEG mounted on a halter and connected to a Track-It Data Logger which measured and logged the voltage harvested by the TEG. We logged the average voltage harvested from 3 animals during 30-min measurement intervals. Animals were not clipped and had approximately a 2 mm hair coat. Data were analyzed with a mixed model using animal as a fixed effect and sampling time as a repeated measure. Throughout the duration of the experiment a sampling rate of 10 Hz was used. Data recovery from the loggers was high (96%) with 4% of data reflecting either open voltage (data logger errors) or no connectivity with the animal (mV <5). After cleaning poor quality data, the average voltage harvested over the data collection period was 53.6 mV. Animals differed significantly ($P < 0.001$) in harvested voltage over the 30-min period and ranged from 45.9 mV to 65.3 mV. Microprocessors typically require a 3.7 V power supply, suggesting additional work on refining the efficiency of energy harvest from TEG is needed before these devices can be used to reliably power wearable precision technologies.

Key Words: thermoelectric generator, renewable energy, precision technologies

T80 Control charts to monitor growth in dairy heifers. P. Turiello*[1], B. Mancilla[1], C. Vissio[1,2], and A. Larriestra[1], [1]*UNRC*, *Río Cuarto, Córdoba, Argentina*, [2]*IDAS UNRC-CONICET*, *Río Cuarto, Córdoba, Argentina*.

There is a need to monitor processes in dairy systems, and there are methods of statistical quality control, like control charts, to effectively do it. Although widely applied in manufacturing industry, control charts have not been much used in animal growth processes. The objective of this study was to build a control chart application as a management tool in a commercial dairy for monitoring and surveillance of the growing process of replacement heifers. Control charts were built from BW data of 295 9-mo old heifers born during the years 2016 to 2018 in a commercial dairy farm in Córdoba province. During the first phase we built the control charts for X-bar and R from 30 samples of 10 heifers each, following the instructions of Montgomery (2005). The center line of the X-bar chart was the average of the sample means (260 kg), and the upper and lower control limits (UCL and LCL, respectively) were calculated with the average of the sample means, the mean range and a constant A_2 (UCL = X-bar-bar + A_2 x R-bar; LCL = X-bar-bar - A_2 x R-bar). For the R chart, the center line was the average of the sample ranges (70 kg), and the UCL and LCL were the average of the sample ranges multiplied by a different constant for each (UCL = D_4 x R-bar; LCL = D_3 x R-bar). The constants A_2, D_4 and D_3 are tabulated values for different sample sizes. Control charts were built and 5 samples out of control were removed after finding assignable causes. From the final graphs the dairy manager can add future sample results and determine that the process is under control if its average is between 238 and 281 kg and the range is between 16 and 124 kg. If the contrary happens the manager can suspect that an assignable cause has occurred, and some investigation should be made and corrective action should be taken to remove the unusual source of variability. These control charts could be used to monitor the growing process of the heifers in the evaluated farm, to detect when some changes are required to take the process back to an in-control state thus reducing the process variability, and to estimate heifer weight among other process variables.

Key Words: quality control, management, replacement program

T81 Concentration levels of butyrylcholinesterase in cows as an indicator of the use of agrochemicals in farms in Colombia. A. Celemin-Sarmiento* and L. Bernal-Bechara, *Animal Science Research Group (ASRG), Animal Science Program, Faculty of Agricultural Sciences, La Salle University*, *Bogotá, Cundinamarca, Colombia*.

The use of agrochemicals in agriculture to increase productivity is well known. However, the impact of these chemical compound to the environment and livestock products is still limited. The aim of the study was to identify if butyrylcholinesterase (BCh) concentration levels in milk serum and blood plasma of cows can be a good predictor for the use of agrochemicals. The study was carried out in 3 agricultural production systems located in the cities: Sopó, Sibaté, and Chía, Cundinamarca state and one production system where agrochemical compounds are not used Sumapaz moorland. Milk samples and blood serum were taken from the cows to determined butyrylcholinesterase concentration. For this, a commercial kit from Wiener lab was used for enzyme determination. Six cows were used as control and 6 cows (2 per farm) that were exposed to agrochemicals products. The data was analyzed as a random completely design by ANOVA and Tukey test was performed to find differences between treatments. Significant differences in blood serum were found between treatments ($P < 0.05$) by BCh. Control reports 233.91 U/L vs Treatment 120.71 U/L. Furthermore, no significant differences in milk serum ($P > 0.05$) was found. Control reports 70.97 U/L vs Treatment 34.07 U/L. Agrochemical compounds was found at low concentration of the enzyme in blood serum compared with the control. Agrochemicals such as organophosphates and carbamates decrease enzyme concentration in the blood plasma of animals exposed to these chemical compounds. So it is suggested to perform the same test in milk in the future to determine the enzymatic concentration of butyrylcholinesterase to evaluate if milk samples can be used for enzymatic butyrylcholinesterase determination.

Key Words: butyrylcholinesterase, milk serum, blood plasma

T82 Associations of milk production and quality with management and housing of robotic milking herds. R. D. Matson*[1], M. T. M. King[1], T. F. Duffield[2], D. E. Santschi[3], K. Orsel[4], E. A. Pajor[4], G. B. Penner[5], T. Mutsvangwa[5], and T. J. DeVries[1], [1]*Department of Animal Biosciences*, *Guelph, ON, Canada*, [2]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*, [3]*Lactanet, Sainte-Anne-de-Bellevue, QC, Canada*, [4]*Faculty of Veterinary Medicine, University of Calgary*, *Calgary, AB, Canada*, [5]*Department of Animal and Poultry Science, University of Saskatchewan*, *Saskatoon, SK, Canada*.

The objective of this study was to identify housing and management factors associated with milk production and quality in robotic milking herds. We visited 197 Canadian robot farms (Western Canada: n = 50, Ontario: n = 77, Quebec: n = 59, Atlantic Canada: n = 11) from April to September 2019 and collected details of barn design and herd management practices. Milk recording data for the 6 mo before farm visits were collected. Farms averaged 111 ± 101 lactating cows, 2.4 ± 1.9 robot units/farm, 47.3 ± 9.1 cows/robot, 36.6 ± 4.9 kg/d of milk, and a herd average SCC of 200,882 ± 94,276 cells/mL. Univariable models were used to screen independent variables (as fixed effects) in mixed-effect linear regression models and variables with $P < 0.25$ were offered to multivariable models, with herd treated as a random effect. A greater number of cows per robot was associated with lesser milk yield; every 10 additional cows was associated with 0.76 kg/d lesser production per cow ($P = 0.02$).The predominant bedding type used was wood products (n = 64; with sand = 52; straw = 41; and other = 28); herds with sand bedding had +1.5 kg/cow/d greater milk production ($P = 0.02$) compared with herds that used wood products. Herds that pushed-up feed > 24 (n = 33) and 12–24 x/d (n = 51) had greater ($P < 0.01$) average production of +2.6 and +1.8 kg/cow/d, respectively, compared with herds that pushed up 1–5 x/d (n = 31). Milk production was associated with region ($P < 0.01$), with Western Canadian farms producing +2.1 and +2.8 kg/cow/d more when compared with Ontario and Quebec farms, respectively. SCC was associated with bedding type; use of sand bedding was associated with a lesser herd average SCC (125,951 cells/mL) when compared with wood products (206,443 cells/mL; $P = 0.005$), straw (269,030 cells/mL; $P < 0.001$), and other bedding types (212,227 cells/mL; $P = 0.02$). The results of this study demonstrate that greater milk production and milk quality are being achieved on robotic milking herds by increasing feed push-up frequency, reducing the stocking density of cows per robot, and using sand to bed their freestalls.

Key Words: automated milking systems, management, production

T83 Does 500 ohms accurately describe the electrical resistance of Idaho dairy cows? R. Norell*[1], M. de Haro Marti[2], J. Wilson[3], M. Chahine[3], J. Packham[4], and K. Kruger[3], [1]*University of Idaho*, *Idaho Falls, ID*, [2]*University of Idaho*, *Gooding, ID*, [3]*University of Idaho*, *Twin Falls, ID*, [4]*University of Idaho*, *Burley, ID*.

By Idaho statue, a 500-Ω shunt resistor is used when investigating stray voltage on dairies and represents the “worst case” body resistance value for adult dairy cows (USDA stray voltage handbook 696, 1992). In recent studies, we have shown that cow electrical resistance (ER) is lower with wet feet, standing in manure, and wet hair coats. Our objective was to compare ER between 3 cow pathways and 2 housing types on commercial dairies. Cow pathways were: muzzle to 4 feet (M4), shoulder to 4 feet (S4) and rear udder to 4 feet (R4). Electrical connections included: cow standing on electrically isolated concrete with 2.5 cm of manure plus a metal mesh on muzzle (M4), metal pipe on wet shoulders (S4), or a metal pipe on wet rear legs + rear udder (R4). These pathways mimic a cow touching a metal waterer with muzzle, touching a manger lockup with wet shoulders and touching parallel parlor pipework with wet rear udder+ legs. Six dairies were enrolled and each provided 15 cows from a freestall barn (FS) and 15 cows from an open lot (OL). Data were analyzed as a randomized complete block design using Proc GLMMIX in SAS. Mean ER (Ω) and % <500 Ω by pathway and by housing were: 254+17, 100; 236+17, 99; and 305+17, 91; 279+14, 96; 251+14, 98; for M4, S4, R4, FS, and OL, respectively. Mean ER was different between cow pathways with R4 being significantly higher ($P < 0.01$) than S4 and M4. ER in FS tended to be higher than ER in OL ($P < 0.06$) but the housing × pathway interaction was not significant ($P < 0.9$). Minimum ER (Ω) by pathway within dairy ranged from 188 to 226; 141 to 183; and 170 to 221; for M4, S4 and R4, respectively. The risk of wet hair coats due to precipitation is greater for OL and motel style FS than gable roofed FS, thus, dairy housing should be considered in stray voltage investigations. All 3 cow pathways and body contact conditions commonly occur on Idaho dairies and measured ER are well below “worst case” from USDA 696. Using a lower value shunt resistor appears desirable to more accurately assess stray voltage risk on Idaho dairies.

Key Words: electrical resistance, stray voltage

T84 A dynamic model to predict herd consistency and milk deliveries of a dairy herd. A. S. Atzori*[1] and A. Gallo[2], [1]*Dipartimento di Agraria, University of Sassari*, *Sassari, Italy*, [2]*Department of Animal Science, Food and Nutrition (DI-*

ANA), Università Cattolica del Sacro Cuore, Piacenza, Italy.

Dynamic modeling applied to dairy farming allows improving the farm decision making process trough prediction of future performances. This work aimed to define a preliminary model structure able to minimize the system complexity and to reach a satisfactory prediction dynamics of a given Mediterranean dairy herd and milk deliveries. The modeling process had the objective of farm output. The modeling process followed a System Dynamics approach and included: i) a preliminary farm survey to gather technical info and data; ii) the development of a Stock and Flow model on Vensim (Ventana Inc.), to mathematically and graphically simulate the dynamic connections among variables; iii) the model evaluation against farm records. The survey provided aggregated monthly records, from Jan 2015 to Dec 2018 (4 years), of consistency, feed supply, milk deliveries, reproduction indexes, mortality and health of herd categories. Average farm characteristics in the same period consisted of 1154 ± 58 milking cows, 205 ± 35.7 dry cows, 1375 ± 45.2 heifers whereas dry matter intake (DMI) and milk yield were on average equal to 24.2 ± 2.4 and 34.0 ± 3.4 kg/d per head, respectively. The model included 25 variables distributed on a closed aging chain of 4 stocks (replacement heifers and open, pregnant and dry cows) and the respective flow rates of calving, breeding, drying off and culling. Initial settings were based on farm values observed in Jan 2015. Two farm inputs (monthly average of consumed feed from lactating cows and conception rate) were included as exogenous variables resulting of fundamental relevance in model predictions. The simulation showed a good accuracy in predicting the oscillating seasonal pattern of historical farm records of milk deliveries. Cow consistency and milk deliveries were predicted with a RMSPE of 3.5% and 7.2% of observed values (being 57% and 68% due to random variability of data), with high accuracy (Cb = 0.90 and 0.97), with good precision (r^2 = 0.68 and 0.72), and with a satisfactory concordance correlation coefficient (0.74 and 0.82), respectively. Model updates should focus on wheater effects on intake and reproduction at farm level.

Key Words: system dynamics, dairy management, weather

T85 Development of an identification system to recognize individual animals based on biometric facial features. R. E. P. Ferreira*[1], L. G. R. Pereira[2,1], T. Bresolin[1], G. J. M. Rosa[1], and J. R. R. Dorea[1], [1]*University of Wisconsin-Madison, Madison, WI*, [2]*Embrapa Dairy Cattle, Juíz de Fora, MG, Brazil.*

In livestock systems, animal identification and traceability are central for food security, data transparency, and consumer trust. A contemporary approach for animal identification is based on image analysis and computer vision, which in general exploit coat color differences among animals using color images. Such method, however, is not efficient for animal species and breeds with homogeneous color patterns. Point cloud deep learning has emerged as a promising machine learning technique that is able to extract features associated with 3D shapes. The objective of this study was to evaluate the efficiency of point cloud deep learning approach for animal identification using 3D images of dairy cattle faces. Images from 9 Holstein cows were acquired at the Dairy Research Cattle Center of the University of Wisconsin-Madison. The images were segmented to remove background and to extract animal faces. This approach was adopted to prevent potential bias toward an overoptimistic model due to recurrent environment noise present in the background of each animal image. A depth sensor (Intel RealSense model D435) was used to acquire 354 images, which were divided into train (n = 214), validation (n = 64), and test (n = 76) sets. A VoxNet point cloud deep learning network was implemented to generate the predictions. Data processing and analyses were implemented in MATLAB 2020a. The accuracy on the testing set was 75%, varying from 50% (for an animal with only 6 images used for training) up to 88.9% (for an animal with 33 images used for training). The results indicate that point cloud deep learning can be a powerful tool for animal identification based on their 3D biometric facial features. Such an approach can be adapted for application in other animal species with homogeneous coat colors and patterns.

Key Words: computer vision, pattern recognition, precision dairy farming

Reproduction 1

T86 Association between automated body condition scores and fertility of Holstein cows. B. Klug[1], D. Manriquez[1], A. Ayyangar[1], A. DeVries[2], S. Granz[3], and P. Pinedo*[1], [1]*Colorado State University*, *Fort Collins, CO*, [2]*University of Florida*, *Gainesville, FL*, [3]*DeLaval*, *Tumba, Botkyrka, Sweden*.

The objective was to analyze the association between body condition score (BCS) and BCS changes (δBCS) at multiple time points during lactation and subsequent fertility. Data were collected from Holstein cows (n = 4,479) in 1 herd in CO, USA (April–December 2019). BCS at 7, 21, and 60DIM and at the d of AI1 (dAI) were obtained from the DeLaval Body Condition Scoring-BCS camera system. BCS (0.1 points intervals/5point scale) were categorized as low (L; mean +SD), intermediate (M; mean ± SD), and high (H; > mean +SD). Cows were also classified by δBCS (gained [GN], no change [NC], mild loss [ML; −0.1 to −0.39], and moderate loss [MOL; < −0.40]) combining multiple time points. Average milk up to 90 DIM (M90) was calculated. Cows were milked 3x and subject to AI following a double OvSynch protocol. Outcomes included pregnancy at AI1 (PAI1) and hazard ratio for conception. Data were analyzed by logistic regression and survival analysis. Calving season (summer vs. other), parity category (1 [n = 2,308]; ≥ 2 [n = 2,171]), and M90 were included in the models. Overall PAI1 was 40.6%. The odds (95%CI) of PAI1 were 1.75 (1.25–2.47) and 1.50 (1.20–1.86); 1.57 (1.20–2.08) and 1.86 (1.49–2.32); 1.93 (1.32–2.84) and 1.56 (1.30–1.87); and 1.48 (1.07–2.06) and 1.77 (1.43–2.20) times greater for cows in H and M vs. cows in L at 7DIM, 21DIM, 60DIM, and at dAI, respectively. PAI1 was associated with δBCS from 7 to 21DIM (GN = 46.2%, NC = 39.8%, ML = 42.0%, MOL = 26.5%; *P* = 004), from 7 to 60 DIM (54.3%, 49.0%, 44.9%, 33.0%; *P* < 0.01), from 21 to 60 DIM (44.6%, 46.4%, 38.9%, 21.4%; *P* < 0.001), and from 21 DIM to dAI (50.1%, 42.9%, 39.3%, 20.1%; *P* < 0.001). The hazard ratio for conception in cows with MOL between 7 to 21DIM, 7 to 60DIM, 7 to dAI, and 21 to 60DIM were 0.49, 0.58, 0.54, and 0.38 relative to cows in GN (*P* < 0.05). In conclusion, low BCS and excessive loss of BCS were associated with lower PAI. Monitoring daily automatic BCS scoring provides valuable information for the assessment of risk of conception.

Key Words: Holstein, BCS, reproduction

T87 Risk factors for early pregnancy loss in primiparous Holstein cows. E. Webster[1], D. Manriquez[1], P. Melendez[2], and P. Pinedo*[1], [1]*Colorado State University*, *Fort Collins, CO*, [2]*University of Georgia*, *Tifton, GA*.

The objective was to identify risk factors associated with early pregnancy loss (EPL) in primiparous Holstein cows in one herd in Colorado. Cows were maintained in a cross-ventilated barn, milked 3×, and subject to AI at about 80 DIM, following a double OvSynch protocol. Data included 5,176 pregnancies in cows conceiving between January 2017 and October 2019. Risk factors considered were: number of inseminations as a heifer (SCH; 1;2; ≥ 3), age at first calving (AFC), calving season (CS), average milk yield up to 100DIM (MY100), number of AI at conception (nAI; 1; 2–3; ≥ 4), season of conception (SCN), and DIM at conception. Pregnancy diagnosis was performed by ultrasonography (US) on d 33 ± 3 after AI and reconfirmed at d 52 ± 3 of gestation. EPL was defined as a positive pregnancy diagnosis at first US followed by a negative diagnosis at reconfirmation. Continuous data were analyzed by ANOVA and binary data by logistic regression. Average (95% CI) EPL was 10.2% (9.21–10.9%). The odds (95% CI) of EPL were 4.46 (2.95–6.76) and 2.70 (2.13–3.42) times greater for SCH ≥ 3 category than for SCH 1 and 2, respectively. The odds of EPL were 1.50 (1.18–1.92) times greater for fall than for winter calvings. Number of breeding was strongly associated with EPL: The odds of EPL were 52 (31–83) and 2.70 (2.19–3.24) times greater for nAI ≥ 4 category than for nAI 1 and nAI 2–3, respectively. The odds of EPL were 1.63 (1.27–2.09); 1.59 (1.21–2.09); and 1.44 (1.14–1.83) times greater for cows conceiving in spring, summer, and fall than for cows conceiving in winter, respectively. The average (SE) DIM at conception in cows having EPL was 121.8 (2.52) d vs. 115.9 (0.85) d in cows that remained pregnant (*P* < 0.01). No significant associations between AFC or MY100 and EPL were determined. In conclusion, EPL was associated with multiple risk factors including SCH, CS, SCN, and DIM at conception. A strong effect was established for nAI, especially for nAI ≥ 4.

Key Words: embryo, mortality, risk

T88 Human chorionic gonadotropin (hCG) dose response 7 days after synchronization of ovulation in lactating Holstein cows I: Ovulatory response and serum progesterone concentrations. E. M. Cabrera*[1], M. R. Lauber[1], E. M. Peralta[1], T. R. Bilby[2], and P. M. Fricke[1], [1]*Department of Dairy Science, University of Wisconsin-Madison*, *Madison, WI*, [2]*Merck Animal Health*, *Kenilworth, NJ*.

Our objective was to assess the effect of treatment with GnRH or 4 increasing doses of human chorionic gonadotropin (hCG) on ovulatory response of a first-wave dominant follicle and subsequent progesterone (P4) concentrations. Lactating Holstein cows (n = 854) were blocked by parity and randomly assigned to receive no treatment (control, C; n = 147), 100 µg of GnRH (Fertagyl, Merck; n = 144), or 1,000 (n = 138), 2,000 (n = 144), 2,500 (n = 142), or 3,300 (n = 139) IU hCG (Chorulon, Merck) 7 d after the second GnRH treatment of an Ovsynch protocol (G2). Ovaries were evaluated with ultrasonography and blood samples were collected immediately before treatment and 7 d later to assess ovulatory response to treatment and serum P4 concentrations. Data were analyzed using the MIXED and GLIMMIX procedures of SAS. Overall, ovulatory response differed (*P* < 0.01) among treatments and was 5%^{a}, 79%^{b}, 77%^{b}, 88%^{c}, 93%^{cd}, and 96%^{d} for C, GnRH, 1,000, 2,000, 2,500 and 3,300 IU hCG treatments, respectively. Ovulatory response did not differ between parities (primiparous vs. multiparous) within each treatment. Primiparous cows had greater (*P* < 0.01) serum P4 concentrations 7 d after G2 than multiparous cows (4.1 vs. 3.6 ng/mL), and cows with double ovulation 7 d after G2 had greater (*P* < 0.01) serum P4 concentrations than cows with single ovulation (4.2 vs. 3.7 ng/mL). Overall, mean serum P4 concentration 7 d after G2 was 3.8 ng/mL and did not differ among treatments. The increase in P4 concentrations from 7 to 14 d after G2 differed (*P* < 0.01) among treatments and was 3.6^{a}, 5.9^{b}, 5.7^{b}, 6.5^{bc}, 7.0^{c}, and 6.5^{bc} ng/mL for C, GnRH, 1,000, 2,000, 2,500 and 3,300 IU hCG treatments, respectively. In conclusion, cows treated with 1,000 IU hCG or 100 µg GnRH 7 d after G2 had similar ovulatory responses (~78%), whereas cows treated with 2,000, 2,500 and 3,300 IU hCG after G2 had increased ovulatory responses (~92%). The increase in serum P4 concentrations from 7 to 14 d after G2 reflected ovulatory responses to treatments.

Key Words: human chorionic gonadotropin (hCG), ovulation, lactating dairy cow

T89 Human chorionic gonadotropin (hCG) dose response 7 days after synchronization of ovulation in lactating Holstein cows II: Pregnancy outcomes and accessory corpus luteum regression. E. M. Cabrera*[1], M. R. Lauber[1], E. M. Peralta[1], T. R. Bilby[2], and P. M. Fricke[1], [1]*Department of Dairy Science, University of Wisconsin-Madison*, *Madison, WI*, [2]*Merck Animal Health*, *Kenilworth, NJ*.

Our objective was to assess the effect of treatment with GnRH or 4 increasing doses of human chorionic gonadotropin (hCG) on pregnancies per artificial insemination (P/AI), pregnancy loss and accessory corpus luteum (CL) regression. Lactating Holstein cows (n = 854) were blocked by parity and randomly assigned to receive no treatment (control, C; n = 147), 100 µg of GnRH (Fertagyl, Merck; n = 144) or 1,000 (n = 138), 2,000 (n = 144), 2,500 (n = 142), or 3,300 (n = 139) IU hCG (Chorulon, Merck) 7 d after the second GnRH treatment of an Ovsynch protocol (G2). Ovaries were evaluated using transrectal ultrasonography immediately before treatment and 7 d later to assess ovulatory response to treatment and accessory CL location relative to the existing CL (ipsilateral vs. contralateral), and pregnancy status was evaluated 32 and 74 d after timed artificial insemination (TAI). Data were analyzed using the GLIMMIX procedure of SAS. Overall, primiparous cows had more ($P < 0.01$) P/AI than multiparous cows at 32 d (57% vs. 48%) and 74 d (52% vs. 43%) after TAI. Overall, treatment did not affect P/AI and was 54%, 49%, 46%, 47%, 57%, and 51% at 32 d and was 48%, 43%, 41%, 44%, 53%, and 46% 74 d after TAI for C, GnRH, 1,000, 2,000, 2,500 and 3,300 IU hCG treatments, respectively. Overall pregnancy loss from 32 to 74 d after TAI did not differ among treatments and was 8%. Cows (n = 108) with bilateral existing or accessory CL due to the Ovsynch protocol or ovulatory treatments were excluded from analyses based on accessory CL location. Overall, regression of accessory CL from 14 to 74 d after TAI for cows that maintained pregnancies differed ($P < 0.01$) between cows with ipsilateral (31%) vs. contralateral (56%) accessory CL; however, regression of accessory CL did not affect P/AI 74 d after TAI. In conclusion, treatment of lactating Holstein cows with GnRH or hCG 7 d after G2 did not affect P/AI or pregnancy loss, and regression was greater for contralateral than for ipsilateral accessory CL.

Key Words: human chorionic gonadotropin (hCG), ovulation, lactating dairy cow

T90 Effect of reusing intravaginal progesterone implant on sexual behavior and pregnancy rate in dairy Gir heifers (*Bos taurus indicus*). R. R. Vicentini[1], A. P. Oliveira[2], R. Veroneze[3], A. Ujita[4], and L. El Faro*[5], [1]*Federal University of Juiz de Fora (UFJF)*, *Juiz de Fora, MG, Brazil*, [2]*Agricultural Research Company of Minas Gerais State (EPAMIG Oeste)*, *Uberaba, MG, Brazil*, [3]*Federal University of Viçosa (UFV)*, *Viçosa, MG, Brazil*, [4]*University of São Paulo (FZEA/USP)*, *Pirassununga, SP, Brazil*, [5]*Institute of Animal Science (IZ)*, *Sertãozinho, SP, Brazil*.

The reuse of progesterone implants on reproductive protocols may be an alternative to reducing expenditures since it does not affect the reproductive success of females. The aim of the study was to observe the effect of the intravaginal progesterone implant reuse on mating receptivity and pregnancy rate in dairy Gir heifers (*Bos taurus indicus*) kept on pasture. The experiment was conducted at Campo Experimental Getúlio Vargas of EPAMIG (Agricultural Research Company of Minas Gerais State, Uberaba, Brazil). Fifty-seven heifers were submitted to fixed-time AI (d 0: 2.0 mg of estradiol benzoate + slow-release progesterone implant impregnated with 1 g of P4 + 19 g of excipient, d 7: 0.52 mg of $PGF_{2\alpha}$; d 9: Intravaginal implant withdrawal + 1 mg estradiol cypionate + 300 IU equine chorionic gonadotropin; d 11: AI + 2.0 mL of GnRH), divided into 2 treatments. G1 (n = 28) received a first-use intravaginal progesterone implant and G2 (n = 29) received a second-use intravaginal progesterone implant. Every 10 min, during a period of 36 h, the mating receptivity behavior of the heifers was recorded. Observations started 12 h after implant withdrawal (d 9) ending at the time of AI (d 11). Transrectal ultrasound shows that all animals had ovulated. The diagnosis of pregnancy was performed 30 d after AI by ultrasound. Statistical analyses were performed using the SAS. Among the 57 heifers observed, 46 (G1: n = 22; G2: n = 24) expressed mating receptivity behavior; however, no sexual reception behavior was observed for 11 animals (G1: n = 6; G2: n = 5). The length of sexual receptivity was similar between the groups ($P > 0.05$), which G1 = 7.20 ± 4.20 h and G2 = 7.6 ± 5.7 h. The non-expression of mating behavior was attributed to the occurrence of silent heat, which can be defined as the occurrence of ovulation with no signs of heat. There was pregnancy rate of 39.28% (n = 11) for G1 and 41.37% (n = 12) for G2, which did not differ statistically ($P > 0.05$). We concluded that the reusing of the progesterone implants apparently did not affect the sexual behavior and pregnancy rate in dairy Gir heifers.

Key Words: reproduction, reproductive protocol, Zebu cattle

T91 Relationships between dietary composition and reproductive outcomes in cattle. T. Davis* and R. White, *Virginia Tech*, *Blacksburg, VA*.

Individual experiments and recent literature summaries suggest links between reproductive outcomes and dietary composition in cattle. However, there is a risk of confounding results in previous meta-analyses focused on a single nutrient because a change in one dietary input requires changes in other nutrients. The objective of this meta-analysis was to evaluate the effects of dietary composition on reproductive efficiency in cattle. A literature search returned 41 previously published papers containing 164 treatment means that were eligible for use. For papers to be used, they needed to report reproductive outcomes, dry matter intake, experimental design details (treatments, design, number of experimental units, measurement SE or SD values, and P-values), and diets with all ingredients and inclusion percentages. Days to ovulation, pregnancy at first artificial insemination, and overall pregnancy rate were the primary reproductive outcomes of interest. Diet composition information of interest included neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), ether extract (EE), non-structural carbohydrate (NSC), lignin and starch percentages. Relationships were analyzed using linear mixed effects models with a random intercept for each study and weighted by the number of experiment units used (due to poor reporting of response variable standard errors). When diet composition information was not reported, it was estimated from commercial feed library data and reported dietary feed inclusion rates. Significant variables were identified through a backward elimination multiple regression approach. Overall pregnancy rate tended to be affected by interactions among the percentages of EE and NDF ($P = 0.095$), ADF and EE ($P = 0.069$), and CP and NSC ($P = 0.023$) in the diet. Days to ovulation was affected the interactions of CP and ADF ($P = 0.010$), CP and starch ($P = 0.005$), and starch and NDF ($P = 0.058$) in the diet. Pregnancy at first AI was not affected by the nutritional factors evaluated here. These data highlight the importance of evaluating nutrient interactions on productive outcomes, rather than focusing on the responses to a single nutrient.

Key Words: cattle, reproduction, meta-analysis

T92 Assessment of the effects of intrauterine dextrose infusion on clinical cure rate, number of health events, culling rate, and reproductive performance in postpartum dairy cows diagnosed with clinical metritis. A. A. Barragan*[1], J. Hamilton[1], E. Hovingh[1], L. Byler[1], M. Martinez[1], S. Bas[2], J. Zug[3], and S. Haan[3], [1]*Department of Veterinary and Biomedical Sciences, Penn State University*, *University Park, PA*, [2]*Phytobiotics Futterzusatzstoffe GmbH Bvd*, *Villa Maria, Córdoba, Argentina*, [3]*Zugstead Farm*, *Mifflintown, PA*.

The objective of this study was to assess the effects of intrauterine dextrose (50%) infusion on clinical cure rate, number of health events, culling rate and reproductive performance in dairy cows diagnosed with clinical metritis (CM). Cows (n = 351) from a dairy farm located in Pennsylvania were screened at 7 ± 3 DIM using a Metricheck device to assess vaginal discharge. Cows that presented a fetid red-brownish watery vaginal discharge (n = 53) were classified as CM cows, blocked by parity and randomly assigned to 1 of 2 groups: 1) CONV (n = 27): 2 injectable (SC) administrations of ceftiofur (6.6 mg/kg; Excede, Zoetis Inc.) 72 h apart; and 2) DEX (n = 26): 3 intrauterine infusions of dextrose (50%; 1 L/cow) 24 h apart. Furthermore, cows that presented a normal vaginal discharge at 7 ± 3 DIM (i.e., dense clear discharge; healthy group; HLT; n = 27) were randomly selected and matched by parity to CONV and DEX cows. Cows were re-screened at 14 ± 3 DIM and 21 ± 3 DIM to assess clinical cure rate. The number of other health events, after CM was diagnosed, and culling rate during the first 60 DIM, and reproductive performance parameters (i.e., DIM to conception, number of services required to conceive) for the first 150 DIM were collected from on-farm computer records. The data were analyzed using the MIXED, GLIMMIX and PHREG procedures of SAS as a randomized complete block design.

There was no difference in clinical cure rate at 14 ± 3 DIM (*P* -value = 0.77; CONV = 50.27 ± 12.49%; DEX = 45.9 ± 12.37%) and 21 ± 3 DIM (*P* -value = 0.96; CONV = 88.05 ± 6.59%; DEX = 88.51 ± 6.35%) between CONV and DEX groups. There was no difference in the number of health events and culling rate in the first 60 DIM between study groups. Cows treated with DEX required more days to become pregnant compared with HLT cows (*P* -value = 0.03; CONV = 95.4 ± 4.92 d; DEX = 102.73 ± 5.68 d; HLT = 81.4 ± 5.68 d). These results suggest that intrauterine infusion of dextrose may have similar outcomes in regard to clinical cure of CM as a conventional antibiotic treatment; however, the latter treatment may decrease the number of days to conception in CM cows.

Key Words: metritis, dextrose, fertility

T93 Pattern of behavioral, physiological, and performance parameters before insemination in dairy cows that became pregnant or not to first service. G. E. Granados*, M. M. Perez, and J. O. Giordano, *Cornell University*, *Ithaca, NY.*

The objective of this study was to compare the pattern of behavioral, physiological, and performance parameters before insemination (AI) in dairy cows that became pregnant or not to their first service (FS). Outcomes (PG = pregnant vs. NP = not pregnant) for FS timed AI after a Double-Ovsynch protocol were collected from lactating Holstein cows [609 primiparous (PP) and 1,010 multiparous (MP)]. Parameters collected from cow-attached and non-attached automated sensors were: milk yield (kg), milk fat (%), protein (%), and lactose (%), milk conductivity (mmHo), BW (kg), activity (AU; arbitrary units), resting time (min), eating behavior (AU), rumination behavior (AU), and reticulo-rumen temperature (°C). Daily data from −14 to 56 DIM was averaged for time periods: −14 to −8, −7 to −3, −2 to 2, 3 to 7, 8 to 14, 15 to 28, 29 to 56 DIM and analyzed by parity using ANOVA with repeated measures. Fixed effects were FS outcome with FS season and lactation group (2 vs 3+ for MP only) as confounders. Cow within FS outcome group was the random effect and object of repeated measures. For PP, milk yield ($P = 0.03$) and temperature ($P = 0.04$) was greater for all time periods for PG than NP cows. No effect FS outcome group or the group by time interaction ($P > 0.10$) was observed for milk fat, protein, fat-to-protein ratio, lactose, conductivity, activity, resting time, BW, eating, and rumination. For MP, there was a group by time interaction ($P < 0.05$) for protein, lactose, temperature, eating, and rumination, a tendency ($P < 0.10$) for milk yield and fat-to-protein ratio, and no effect ($P > 0.10$) for fat, conductivity, activity, resting time, and BW. Protein (15 to 56 DIM), lactose (0 to 7 DIM), eating (3 to 7 DIM), and rumination (3 to 7 DIM) were greater for PG than NP and temperature (3 to 28 DIM) was lower for NP than P. We conclude that there are differences in behavioral, physiological, and performance parameters before insemination in lactating dairy cows that become pregnant or not to FS but differences are parity-specific.

Key Words: sensor, pregnancy, dairy cow

T94 Association between age at first calving and economic performance of replacement dairy heifers. M. Masello*[1], M. M. Perez[1], G. E. Granados[1], M. L. Stangaferro[1,2], B. Ceglowski[2], M. J. Thomas[2], and J. O. Giordano[1], [1]*Department of Animal Science, Cornell University*, *Ithaca, NY*, [2]*Dairy Health and Management Services*, *Lowville, NY.*

Our objective was to evaluate the association between age at first calving (AFC) and the economic performance of Holstein replacements. Nulliparous heifers (n = 1,144) from 3 farms were eligible for AI at 368 ± 10 d of age [beginning of the AI period (AIP)]. Sexed semen was used for first AI and conventional semen for second and greater AI. After calving, heifers were retrospectively assigned to 1 of 3 groups based on AFC tertiles within farm: Low (n = 391): 20.2–21.8 mo, Medium (n = 376): 21.4–22.8 mo, and High (n = 377): 22.1–28.7 mo. Individual heifer costs and revenues were collected for 15 mo after the beginning of the AIP [rearing period (RP) and part of the first lactation (FL)]. Total cash flow (CF) was the aggregation of RP reproductive cost (RC), RP feed cost (FDC), RP fixed cost (FXC), calf value (CFV), FL income over feed cost (IOFC), FL replacement cost (RPLC), and FL FXC. Binomial and quantitative data were analyzed by logistic regression and ANOVA, respectively. All results are in Table 1. Differences in AFC among groups were explained by differences in age and P/AI at first service. All RP costs were greater with increasing AFC. Similarly, most FL parameters favored heifers with low

Table 1 (Abstr. T94). Reproductive and economic outcomes

Item	Low	Medium	High	*P*-value
Age 1st AI (d)	371 ± 0.5^{a}	376 ± 0.5^{a}	378 ± 0.5^{b}	<0.01
P/AI 1st AI (%)	95.9^{a}	33.2^{b}	0.27^{c}	<0.01
RP (d)	276 ± 5.7^{a}	301 ± 5.7^{b}	352 ± 5.7^{c}	<0.01
FL (d)	180 ± 5.7^{a}	155 ± 5.7^{b}	104 ± 5.7^{c}	<0.01
Rearing ($/heifer)				
RC	39.9 ± 0.9^{a}	57.0 ± 0.9^{b}	94.6 ± 0.9^{c}	<0.01
FDC	533 ± 13^{a}	587 ± 13^{b}	705 ± 13^{c}	<0.01
FXC	235 ± 4.8^{a}	256 ± 4.8^{b}	300 ± 4.8^{c}	<0.01
Total RP cost	807 ± 22^{a}	900 ± 22^{b}	1,099 ± 22^{c}	<0.01
First lactation ($/heifer)				
CFV	97.8 ± 1.8^{a}	96.7 ± 1.8^{ab}	92.5 ± 1.8^{c}	0.05
IOFC	980 ± 58^{a}	850 ± 58^{b}	583 ± 58^{c}	<0.01
RPLC	68.9 ± 13	60.1 ± 13	51.2 ± 13	0.58
FXC	557 ± 18^{a}	480 ± 18^{b}	321 ± 18^{c}	<0.01
Total FL profit	454 ± 45^{a}	408 ± 45^{a}	304 ± 45^{b}	<0.01
CF 15 mo	−354 ± 63^{a}	−492 ± 63^{b}	−795 ± 63^{c}	<0.01

vs. high AFC whereas heifers with medium AFC had intermediate performance. We conclude that the profitability of replacement heifers during a 15-mo period after the beginning of the AIP increases as AFC decreases.

Key Words: profitability, age at first calving, dairy heifer

T95 Characterization of pregnancy associated glycoproteins (PAG) and progesterone (P4) as a predictor of twins and conceptus loss in high-risk pregnancy Holstein cows. P. M. Peixoto*[1], A. M. Hubner[1], L. L. Cunha[1,2], W. M. Coelho Jr[1], K. G. Pohler[3], N. W. Dias[4], V. R. G. Mercadante[4], I. F. Canisso[1,2], and F. S. Lima[1,5], [1]*Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois*, *Urbana, IL*, [2]*Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois*, *Urbana, IL*, [3]*Department of Animal Science, Texas A&M University*, *College Station, TX*, [4]*Department of Animal and Poultry Sciences, Virginia Tech University*, *Blacksburg, VA*, [5]*Department of Population Health and Reproduction, University of California*, *Davis, CA*.

The objective of this study was characterizing plasma concentration of PAG and P4 as predictor of twins and conceptus loss in high-risk pregnancy Holstein cows. High risk pregnancy (HR) were characterized using transrectal ultrasonography 37 d post AI based on the following criteria: small embryo size (SE, embryo <15 mm, n = 10), slow heartbeat (SH, < 60 beats per minute, n = 11), extra amniotic membrane (EM, additional amniotic membrane, n = 3). A cohort of twins (TW, n = 41) diagnosed at d 37 post AI was also enrolled. Each HR and TW cow was paired with a same parity cow carrying a normal singleton at d 37 post AI (CON, n = 65). Blood samples were collected to measure PAG and P4 at 37, 44, and 51 post AI. Statistical analysis was performed using ANOVA, logistic regression and receiver operation characteristics (ROC) with JMP. Pregnancy loss at d 51 post AI was greater ($P < 0.01$) in HR than CON and TW (CON = 1.5%; HR = 87.5%; TW = 12.2%). Concentration of PAG at d 37 post AI did not differ ($P = 0.75$) among groups (CON = 5.3 ± 0.7; HR = 4.8 ± 1.2; TW = 4.0 ± 0.9 ng/mL). Concentration of P4 at d 37 post AI was greater in TW than HR and CON, and lower ($P < 0.01$) in HR than CON cows (CON = 7.0 ± 0.3; HR = 5.9 ± 0.4; TW = 8.4 ± 0.3 ng/mL). Regression and ROC analysis for PAG at d 37 post AI did not find a threshold to predict pregnancy loss ($P = 0.24$) or twins ($P = 0.30$). Regression and ROC analysis for P4 at d 37 post AI found that a threshold of 6.5 ng/mL predicted ($P < 0.01$) pregnancy loss with an area under the curve (AUC) of 0.65, and threshold of 7.2 ng/mL predicted ($P < 0.01$) twins with AUC of 0.70. In summary, pregnancy loss and twins were predicted with only moderate accuracy by P4 concentration at d 37 post AI and PAG concentration at d 37 post AI were not able to generate a threshold to predict pregnancy loss and twins in Holstein lactating cows

Key Words: pregnancy loss, twins, progesterone

T96 Prediction of pregnancy probability at first service in multiparous dairy cows based on early lactation data. M. Stangaferro*[1,2], J. Giordano[2], M. Toledo[3], and M. Wiltbank[3], [1]*Dairy Health and Management Services*, *Lowville, NY*, [2]*Cornell University*, *Ithaca, NY*, [3]*University of Wisconsin-Madison*, *Madison, WI*.

Objectives were to identify factors associated with first service pregnancies per AI (P/AI) and develop models to predict the probability of pregnancy in dairy cows. Data from multiparous Holstein cows (n = 410) from 2 farms that received TAI at 80 ± 3 DIM after the Double-Ovsynch (DO) protocol were used. Data included calving season, health records, uterine cytology, daily milk yield, biweekly milk components, and repeated body condition score (BCS), BW, and progesterone concentration (P4) measurements. Bivariate analysis with logistic regression was used to test associations between predictors and AI outcome (pregnant or not), and variables with $P < 0.20$ were retained for prediction. Only the variable with the smallest p-value was included if 2 variables were correlated ($r \geq 0.6$, $P < 0.05$). Logistic regression models for prediction were developed by backward elimination (predictor removed if $P > 0.10$) and lowest AIC value. Predicted probabilities for each cow were obtained to generate ROC curves. First service P/AI was affected by calving season [$P = 0.03$; Cold = 42.0%, Warm = 55.7%; OR = 1.64 (1.05–2.56)], displaced abomasum [$P = 0.03$; n = 50.0%, Y = 14.3%; OR = 0.18 (0.04–0.85)], mastitis [$P < 0.01$; n = 54.3%, Y = 31.3%; OR = 0.40 (0.23–0.68)], lameness [$P = 0.08$; n = 50.7%, Y = 28.6%; OR = 0.46 (0.19–1.08)], cytological endometritis [$P < 0.01$; n = 54.6%, Y = 35.9%; OR = 0.52 (0.32–0.85)], P4 ≥ 2 ng/mL at last PGF of DO [$P < 0.01$; n = 24.1%, Y = 52.8%; OR = 3.44 (1.71–6.91)], P4 < 0.6 ng/mL at GnRH before TAI [$P = 0.02$; n = 15.4%, Y = 49.9%; OR = 7.53 (1.42–39.93)], fat yield at wk 4 of lactation [$P = 0.03$; OR = 0.93 (0.87–0.99) for every 100g increased in fat yield], and lowest BCS value between calving and TAI [$P < 0.01$; OR = 9.75 (3.60–26.47) for every unit increased in lowest BCS value between calving and TAI]. The AUC obtained with the ROC curve for the predicted probabilities was 0.76 ($P < 0.01$), with 78% sensitivity and 63% specificity using a cut-off > 0.46. We conclude that the likelihood of pregnancy at first service in multiparous cows could be approximated by using early lactation health records, body condition, hormone concentrations, and calving season data.

Key Words: prediction, pregnancy, first service

T97 Effect of gonadotropin-releasing hormone (GnRH) seven days before pre-synchronization with simultaneous prostaglandin $F_{2\alpha}$ and GnRH on reproductive outcomes in Holstein dairy cows. A. Hubner*[1,2], P. Peixoto[2], J. Hillesheim[3], I. Canisso[1,2], and F. Lima[4], [1]*Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois*, *Urbana, IL*, [2]*Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois*, *Urbana, IL*, [3]*Lena Veterinary Clinic*, *Lena, IL*, [4]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California*, *Davis, CA*.

The objective of this study was to improve a first-service synchronization program that was designed for farms that wish to reduce the number of treatment days of typical GnRH pre-synchronization programs. Our hypothesis was that adding a GnRH 7 d before simultaneous treatments of GnRH and $PGF_{2\alpha}$ in a program where OvSynch started 7 d after those simultaneous treatments of GnRH and $PGF_{2\alpha}$ would increase the percentage of cows having a CL at the start of the breeding OvSynch and thereby increase pregnancies per AI. Lactating dairy cows (n = 406) were blocked by parity and randomly assigned to control or treatment. Control cows (n = 205) received a concurrent $PGF_{2\alpha}$ and GnRH 7 d before the first GnRH of OvSynch (GnRH-7d-PGF2α-56 h-GnRH-16h-AI). Treated cows (n = 201) received GnRH followed by concurrent $PGF_{2\alpha}$ and GnRH in 7 d followed by the start of OvSynch in 7 d. Pregnancies/AI were similar in controls vs. treated cows on Day 32 (33% ± 3.4% vs. 35% ± 3.4%, $P = 0.75$) and Day 67 (32% ± 3.3% vs. 32% ± 3.4%, $P = 0.88$), respectively. There was tendency for an interaction between treatment and parity, with multiparous cows tending to have more pregnancies/AI to the program with the additional GnRH ($P = 0.09$). Percentage of cows ovulating to the GnRH of the simultaneous treatments of GnRH and $PGF_{2\alpha}$ was not different between control and treatment, nor was there a difference in the percentage of cows starting the breeding OvSynch with a CL. In summary, addition of a GnRH treatment 7 d before simultaneous treatments of GnRH and $PGF2_{\alpha}$ administered 7 d before the start of OvSynch did not improve ovulatory response to the GnRH of the simultaneous GnRH and $PGF_{2\alpha}$, nor did it increase pregnancies per AI.

Key Words: presynchronization, timed AI, anovular

T98 Factors affecting pregnancy associated glycoprotein (PAG) and pregnancy specific protein B (PSPB) profiles in plasma during early pregnancy. R. C. Doyle*[1,2], J. Kenneally[1], M. C. Lucy[1], and S. T. Butler[1], [1]*Teagasc*, *Fermoy, Cork, Ireland*, [2]*University of Missouri*, *Columbia, MO*.

The objective was to examine the effect of cow genetic background (GT: elite Holstein-Friesian, national average Holstein-Friesian (NA), Jersey), fertility index (FI; low < €61, medium €61-€99 and high ≥ €100), milk index (MI; low < €26, medium €26-€40 and high ≥ €41), milk yield (MY), feeding treatment (low grass allowance (LGA), control, high concentrate (HC)) and parity (1, 2, 3+) on plasma pregnancy associated glycoprotein (PAG) and pregnancy specific protein B (PSPB) on d 23, 25 and 28 post-insemination (n = 76 cows). A standard curve was generated for both assays using a pooled sample of plasma collected from pregnant cows serially diluted with plasma from non-pregnant cows (100%, 50%, 25%, 12.5%, 6.25% and 0%). Transformed PAG and PSPB concentrations were analyzed using generalized linear mixed models (results presented as back-transformed LS means and 95% CI). An effect of feeding treatment ($P = 0.0035$) was observed on the PAG fold change from d 23 to 25 post insemination (5.9 [4.9, 6.9], 3.9 [2.7, 5.2] and 3.8 [2.8, 4.7] for LGA, control and HC, respectively). There was greater fold change from d 23 to 25 with increasing parity number (−1.7 [-10.1, 6.7], 6.3 [4.8, 12.3] and 17.4 [8.1, 26.7] for parity 1, 2 and 3+, respectively; $P = 0.02$) and FI (−1.4 [-9.1, 6.3], 0.53 [-8.3, 9.4] and 22.9 [12.7, 32.9] for low, medium and high FI, respectively; $P < 0.0003$) on PSPB concentrations. There was lesser fold change from d 25 to d 28 on PSPB concentrations with increasing MI (2.6 [2.0, 3.21], 1.6 [0.5, 2.6] and 1.0 [0.1, 1.9] for low, medium and high MI, respectively; $P = 0.01$), MY (3.10 [2.0, 4.2], 2.7 [1.8, 3.7], 1.2 [0.3, 2.1] and −0.1 [-1.5, 1.2] for 25th, 50th, 75th and upper quartiles, respectively; $P = 0.006$)and GT (2.8 [1.7, 3.8], 2.0 [0.74, 3.2] and 0.4 [-0.8, 1.6] for elite, NA and Jersey, respectively; $P = 0.03$). In conclusion, PAG was affected by feeding treatment and PSBP was affected by parity, FI, MI, MY and GT. Multiple factors affect PAG and PSPB signal variation between d 23 and 28 post-insemination with PSPB appearing to be more sensitive to the parity, genetics and MY of the cow.

Key Words: embryo development, genetic merit

T99 Characterization of ano-genital distance and its relationship to fertility in Holstein heifers. J. E. Carrelli*[1], M. Gobikrushanth[1], M. G. Colazo[2], and D. J. Ambrose[2,1], [1]*Department of Agricultural, Food, and Nutritional Science, University of Alberta*, *Edmonton, AB, Canada*, [2]*Livestock Systems Section, Alberta Agriculture and Forestry*, *Edmonton, AB, Canada*.

Improving female fertility has become a key breeding goal in the dairy industry, where various measures of reproductive fitness have been incorporated in merit indices worldwide however; due to low heritability, the genetic progress for reproductive traits has been slow. With recent industry advancements, additional traits with the potential to complement current traits are beginning to be reviewed and implemented into selection programs. Ano-genital distance (AGD, the distance from the center of the anus to the base of the clitoris) is a sexually dimorphic trait believed to reflect prenatal androgenization during a heifer's reproductive programming window. In a preliminary report, AGD in primiparous and multiparous cows was inversely related to fertility and exhibited moderate heritability. Thus, AGD may be a useful reproductive phenotype for the future of genetic selection. The objective of this study was to (1) characterize AGD and (2) determine if the same inverse relationship exists between AGD and fertility in nulliparous heifers. AGD was measured in 671 Holstein heifers across western Canada and data was analyzed using UNIVARIATE, MIXED, GLIMMIX and LIFETEST procedures of SAS. Mean age at AGD measurement was 485 ± 78 d, AGD was normally distributed with a mean (±SD) of 114 ± 11.5 mm, ranging from 81 to 148 mm. Mean AGD was used to categorize heifers into short (<114 mm) and long (≥114 mm) AGD groups, and associations with fertility were determined. Heifers with short-AGD achieved pregnancy earlier (444 ± 8.5 vs. 457 ± 8.4 d; $P < 0.01$) and more efficiently (1.6 ± 0.13 vs. 1.8 ± 0.13 times bred; $P = 0.02$) than those with long-AGD. In addition, heifers with short-AGD had greater pregnancy to first artificial insemination than heifers with long-AGD (62.2 ± 5.7 vs. 52.5 ± 5.8%; $P = 0.01$) however; the rate of pregnancy up to 540 d of age did not differ between short- and long-AGD groups (95 vs. 95%; $P = 0.80$). In summary, an inverse relationship between AGD and fertility measures in nulliparous heifers is apparent, strengthening the potential for AGD to be used as a fertility trait in future selection programs. Studies using a larger population are needed to corroborate these findings.

Key Words: heifer fertility, reproductive phenotype, genetic improvement

T100 Repeatability of ano-genital distance measurements at different stages of the estrous cycle. I. Rajesh*[1], M. Gobikrushanth[1], J. E. Carrelli[1], and D. J. Ambrose[1,2], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta*, *Edmonton, AB, Canada*, [2]*Livestock Research Section, Alberta Agriculture and Forestry*, *Edmonton, AB, Canada*.

The fertility of dairy cows may be improved by genetic selection for novel fertility traits. In a recent report from our lab, ano-genital distance (AGD; the distance from the center of the anus to the base of the clitoris), a novel reproductive phenotype, in Canadian Holstein cows was inversely associated with fertility and had moderate heritability. In addition to heritability, repeatability (i.e., the closeness of the measurements taken at different time points by the same person) is important for AGD to qualify as a potential fertility trait. The objective of this study, therefore, was to determine if AGD is repeatable at the different stages of the estrous cycle. The AGD was measured in triplicate (the mean value used), in 20 Canadian Holstein cows that were subjected to estrous synchronization using a progesterone-device-based ovulation synchronization protocol. The ovaries were subjected to transrectal ultrasonography and blood samples were collected for plasma progesterone quantification, every other day, starting from first ovulation until the subsequent ovulation. The stages of the estrous cycle were classified as proestrus, estrus, metestrus, and diestrus based on the size of corpus luteum and plasma progesterone concentration. Data were analyzed using the MIXED and CORR procedure of SAS. The average AGD measurements at different stages of the estrous cycle within the same animal did not differ (AGD at proestrus, estrus, metestrus, and diestrus were 130.81 ± 2.1, 131.19 ± 2.1, 131.17 ± 2.1, and 131.38 ± 2.1, respectively; $P = 0.99$) and the correlation between them was very high ($r = 0.98$; $P < 0.01$). In summary, AGD was not affected by the stages of the estrous cycle and AGD measurements were highly repeatable. Our findings further strengthen the reasons for pursuing AGD as a potential fertility trait.

Key Words: reproductive phenotype, genetic selection, heritability

T101 Gene expression in the uterus of heifers challenged with lipopolysaccharide. J. Alvarado-Rincón, G. de Avila Ferronato, A. Stein Maffi, A. Amaral Barbosa, R. Klaus, J. Feijó, F. Del Pino, G. Bueno Luz, B. Garziera Gasperin, R. Gianella Mondadori, E. Schmitt*, V. Rohrig Rabassa, M. Nunes Correa, and C. Cassal Brauner, *Núcleo de Pesquisa, Ensino e Extensão em Pecuária, UFPel*, *Pelotas, RS, Brazil*.

Even in subclinical form, infectious or metabolic diseases can cause an increase in lipopolysaccharide (LPS) in the bloodstream, which has been negatively associated with fertility. The aim of the study was to investigate the expression of genes associated with the uterine environment quality and inflammation in the uterus of heifers challenged with LPS. Beef heifers (n = 16) *Bos taurus*, healthy, 14 mo of age, in confinement system, were submitted to a hormonal protocol for follicular wave synchronization (Day-14: PGF2α; Day 0: insertion P4 device, Estradiol benzoate, and PGF2α). On d 0 the LPS group (n = 8) received 2 intravenous applications of 2 mL of saline solution containing 0.5 μg/kg of BW of

LPS (*E. Coli*, Sigma Aldrich Missouri, USA) with an interval of 24h and the control group (n = 8) received 2 intravenous applications of 2 mL of saline solution with the same interval. The body temperature was measured at 0, 4, 24, and 28h. All animals were slaughtered on d 5, when uterine samples were collected. Relative expression was calculated using the *RN18S1* and *GAPDH* genes as housekeeping, and genes associated with LPS recognition (*TLR4*), inflammatory response (*IL-1β* and *TNF*) and quality of the uterine environment (*PTGS2* and *NANOG*) were evaluated. The body temperature of the LPS group was greater than the control, at 4h (39.9 ± 0.2°C *vs* 39.1 ± 0.1°C, $P < 0.05$) and at 28h (40.4°C ± 0.3°C *vs* 39.3 ± 0.1°C, $P < 0.05$). This result indicates that the dose of LPS used was able to generate a systemic inflammatory response 4h after each challenge. In addition, the LPS generated a decrease of 53% in the relative expression of *PTGS2* (LPS: 0.47 ± 0.18 *vs* Control: 1.0 ± 0.13, $P = 0.024$) and of 92% for *NANOG* (LPS: 0.08 ± 0.07 *vs* Control: 1.0 ± 0.4, $P = 0.020$). However, *TLR4, TNF,* and *IL-1β* were not affected by LPS. This is the first in vivo study to use this methodology (dose and interval) and it was found that directly or indirectly, the systemic challenge with LPS altered the expression of genes involved in the synthesis of E2 prostaglandin and cellular pluripotency, important mediators in the implementation of embryo and in the endometrial regeneration process.

Key Words: fertility, inflammation, LPS

T102 Association between postpartum health and subsequent detection of spontaneous estrus by activity monitors in dairy cows. T. C. Bruinjé*[1], E. I. Morrison[1], R. Couto Serrenho[1], E. S. Ribeiro[2], D. Renaud[1], and S. J. LeBlanc[1], [1]*Department of Population Medicine, University of Guelph*, *Guelph, ON, Canada*, [2]*Department of Animal Biosciences, University of Guelph*, *Guelph, ON, Canada*.

Our objective was to investigate associations between postpartum health and the probability of detection of spontaneous estrus between 50 and 75 d in milk by automated activity monitors (AAM) in dairy cows. In this observational study, Holstein cows (n = 454) from 2 dairy farms in Ontario were enrolled at wk −3 relative to parturition. Body condition score (BCS) was assessed at wk −3 and 9, and lameness at wk −3, 3, and 7. Total calcium (Ca), haptoglobin (Hp), and nonesterified fatty acids (NEFA) were measured in serum at d 2 and 6 (±1; SD). At d 4, 8, 11, and 15 (±1), blood β-hydroxybutyrate (BHB) concentrations were measured and the occurrence of metritis (brown, watery, foul-smelling vaginal discharge with or without fever) assessed. Cows were examined for purulent vaginal discharge (PVD) and subclinical endometritis (≥6% polymorphonuclear cells in endometrial cytology and no PVD) at wk 5. Herds used detection of estrus by AAM with no hormonal intervention for first AI until d 75. Metabolites were categorized based on ROC curve cutpoints associated with PVD. Detection of estrus between d 50 and 75 was analyzed in a multivariable logistic regression model, including parity, health variables, interactions, and herd as a random effect. Common health disorders were hypocalcemia (Ca ≤2.1 mmol/L; 60% of cows) and high Hp (≥0.7 g/L; 37%) at d 2, high NEFA (≥0.5 mmol/L) at d 6 (47%), hyperketonemia (BHB ≥1.3 mmol/L) at any sample (25%), subclinical endometritis (24%), PVD (19%), and metritis (14%). Overall, 61% of cows were detected in estrus between d 50 and 75. The odds of estrus detection were reduced in cows with vs. without PVD (46 vs. 66%; $P < 0.01$), in cows with high Hp at both d 2 and 6 vs. at least one sample with low Hp (43 vs. 64%; $P = 0.01$), or in cows with BCS ≤2.75 vs. ≥ 3.00 at wk 9 (50 vs. 62%; $P = 0.05$). In the 32% of cows without these risk factors, 72% were detected in estrus vs. 56% in cows with all 3 risk factors ($P < 0.01$). Identifying variables associated with reduced odds of estrus detection by AAM will allow refinement of reproductive management decisions.

Key Words: activity monitoring, reproduction, postpartum health

Ruminant Nutrition: General 2

T103 Fumonisin esterase degrades fumonisins in lactating dairy cows. A. Gallo[1], A. Minuti[1], B. Doupovec[2], J. Faas*[2], G. Bichl[2], D. Schatzmayr[2], and E. Trevisi[1], [1]*Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, Piacenza, Italy*, [2]*BIOMIN Research Center, Tulln, Austria.*

Fumonisins (FBs) are commonly detected mycotoxins in dairy diets. Despite a lack of studies on the in vivo effects of FBs in dairy cows, it is known from in vitro experiments that the rumen has little ability to degrade FBs. Fumonisin esterase FumD catalyzes the degradation of FBs in the gastrointestinal tract, producing less harmful hydrolyzed or partially hydrolyzed fumonisins (HFBs and pHFBs). The aim of this trial was to test the efficacy of the enzyme when fed to lactating dairy cows. In a 3 × 3 4 times replicated Latin square design, a total of 12 Holstein cows was used. Treatments were (1) an uncontaminated TMR (CTR), (2) a TMR contaminated with FBs (1.1 mg/kg) (MTX), or (3) the MTX TMR supplemented with a mycotoxin deactivator product containing FumD (MDP) (35 g/animal/day) (MTX+MDP). Each experimental period consisted of a 3-wk treatment period followed by a 2-wk clearance period. Ruminal fluid samples were collected at 5 h after the morning feeding on d 21 of each experimental period via a stomach tube. Fecal samples were taken before the start (d 0) and at d 21. Data were analyzed using SPSS 22.0. If data met the requirements, a one-way ANOVA followed by a Games-Howell post hoc test was used, if not a nonparametric Kruskal-Wallis test was conducted. Upon administration of FumD, the sum of FBs (FB1+FB2+FB3) decreased and the sum of HFBs (HFB1+HFB2+HFB3) and pHFBs (pHFB1+pHFB2+pHFB3) increased in ruminal fluid (d 21: sum of FBs: CTR 58 μmol/L, MTX 508, MTX + MDP 265 μmol/L, $P < 0.01$, sum of HFBs and pHFBs: CTR 57 μmol/L, MTX 115 μmol/L, MTX + MDP 287 μmol/L, $P < 0.01$). In feces there was no treatment effect visible at d 0 of the experiment. However, treatments differed significantly after 21 d (sum of FBs: CTR 2149 μmol/L, MTX 15297 μmol/L, MTX + MDP 7605 μmol/L, $P < 0.01$; sum of HFBs and pHFBs: CTR 2315 μmol/L, MTX 2804 μmol/L, MTX + MDP 8351 μmol/L, $P < 0.01$). Consequently, in both ruminal fluid and feces, the feeding of the enzyme in the MTX+MDP treatment decreased the sum of FBs in comparison to the MTX treatment while increasing the sum of the less harmful metabolites HFBs and pHFBs, showing its efficacy in degrading fumonisins.

Key Words: mycotoxins, fumonisins, fumonisin esterase

T104 Effect of potassium sorbate application rate and timing of application on aerobic stability of corn silage. J. D. Stypinski*, C. A. Mellinger, X. Liu, N. A. Moyer, and L. Kung Jr., *University of Delaware, Newark, DE.*

Potassium sorbate (PS) is a known for its ability to inhibit yeasts that usually initiate aerobic spoilage in silages. The objective of this study was to determine an effective dose rate of PS for improving the aerobic stability (AS) of corn silage and to determine if the time of application (at ensiling or at silo opening) affected its efficacy. Potassium sorbate was added to freshly chopped whole plant corn at 0, 100, 250, or 500 g/t of fresh weight. Five individually replicated 7.5 L bucket silos were prepared for each of the 4 treatments. The silos were stored at 21°C and opened after 90 d of ensiling. Upon silo opening, portions of the untreated silages were treated with similar levels of PS as described. About 2 kg of silages treated with PS at ensiling or at opening were exposed to air (21°C) by placing them in clean silos that had a thermocouple wire placed in the geometric center of the silage mass. Temperature was measured every 15 min and AS was defined as the time it took before a 3°C rise in the baseline temperature of each silo. Data were analyzed as a completely randomized design using JMP and significance was declared at $P < 0.01$. Adding PS at the time of ensiling had minimal effects on fermentation end products (lactic and acetic acid and ethanol) after ensiling for 90 d. Increasing levels of PS numerically decreased the numbers of yeasts in silages with addition of the 500 g dose (log 3.79 cfu/g of silage) being different ($P < 0.01$) from untreated silage (log 4.37 cfu/g). Similarly, increasing levels of PS at ensiling increased AS with the highest dose (225 h of AS) being different ($P < 0.01$) from untreated silage (71 h of AS). Adding PS at silo opening, resulted in similar numerical improvements to aerobic stability similar to when it was added at ensiling. Treatment at the highest dose to silage after ensiling resulted in the greatest improvement in AS (>310 h) compared with all other treatments. In this study, a dose of at least 500 g of PS/t of fresh forage weight was needed to statistically improve the AS of silages and it did not matter if silage was treated at ensiling or after silo opening.

Key Words: silage, potassium sorbate, spoilage

T105 Ruminal dry matter, crude protein, and neutral detergent fiber degradation of whole-plant soybean silage. L. G. Ghizzi*[1,3], T. A. Del Valle[2], C. Heinzen[3], M. R. Pupo[3], L. F. Ferraretto[3], and F. P. Rennó[1], [1]*University of São Paulo, Pirassununga, SP, Brazil*, [2]*University of Pampa, Itaqui, Rio Grande do Sul, Brazil*, [3]*University of Florida, Gainesville, FL.*

This study aimed to evaluate the effects of molasses (MOL) on whole-plant soybean silage (WPSS) with different phenological stages (PS) on the ruminal degradation kinetic parameters and effective degradability (ED) of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF). Treatments were arranged in a factorial 3 × 2 with a completely randomized design to evaluate both PS of WPSS [R5 (beginning seed), R6 (full seed), and R7 (beginning maturity)] and MOL (0 and 40 g/kg fresh forage). Whole-plant soybean silage was made into 60 experimental silos with 650 kg/m^3 bulk density and stored at room temperature for 90 d. Degradation parameters and ED of DM, CP, and NDF, were determined with the in situ nylon bags method by ruminal incubation in 3 lactating Holstein cows for 0, 3, 6, 12, 24, 48, 72, 120, and 240 h. Ruminal rapidly (fraction A), slowly (fraction B) and undegraded (fraction C) fractions, rate constant of degradation (kd), and ED (at ruminal passage rate of 0.03 h^{-1}) were calculated using the exponential model of Ørskov and McDonald (1979) using PROC NLMIXED of SAS 9.3. In general MOL increased ($P \leq 0.002$) fraction A and ED, decreased ($P \leq 0.016$) fraction C without effecting ($P \geq 0.131$) fraction B and kd for DM, CP, and NDF except fraction B for DM which decreased ($P = 0.037$). Increasing PS increased ($P < 0.001$) fractions A and B, kd, and ED while decreasing ($P < 0.001$) fraction C for DM and CP. Whereas increasing PS increased ($P < 0.001$) fraction A and ED and decreased ($P < 0.001$) fractions B and C and kd for NDF. The magnitude of effect for MOL was lower with increasing PS. Although greater results were observed in early PS, MOL can be used in WPSS to increase DM, CP, and NDF degradation regardless of PS. Additionally, R7 optimizes ruminal DM, CP, and NDF degradation.

Key Words: additive, in situ degradation, legume ensiling

T106 Assessing milk response to branched-chain volatile fatty acids. K. E. Mitchell*[1], M. T. Socha[2], L. E. Moraes[1], Y. Roman Garcia[1], and J. L. Firkins[1], [1]*The Ohio State University, Columbus, OH*, [2]*Zinpro Corporation, Eden Prairie, Minnesota.*

Supplemental branched-chain volatile fatty acids (BCVFA) and valerate (i.e., "isoacids") have improved both NDF digestibility and feed efficiency in ruminants. Cellulolytic bacteria require BCVFA to synthesize branched-chain amino acids (BCAA) and branched-chain fatty acids because they cannot uptake or synthesize BCAA de novo. Many studies provided isobutyrate (IB), 2-methylbutyrate (MB), isovalerate (IV), and valerate (V) altogether without exploring optimal combinations. Our objective was to determine a combination of isoacids that is optimal for milk production. Sixty (28 primiparous and 32 multiparous) lactating Jersey cows (106 ± 54 DIM) were blocked and randomly assigned to 4 treatments: 1) control (CON) with no isoacids, 2) MB (12.3 mmol/kg DM), 3) IB+MB (12.6, and 7.7 mmol/kg DM), or 4) all 4 isoacids (7.3, 6.2, 4.2, and 5.1 mmol/kg DM IB, MB, IV, and valerate). All cows were fed CON for a 2-wk period, then randomly assigned within block to treatments for 8 wk (n = 15). Milk samples from 4 milkings per week were analyzed by DHI (Columbus, OH). Data were analyzed with PROC MIXED (v. 9.4, SAS Institute 2015) with the random effect of block and fixed effects of treatment, week, and parity. Data from the wk 2 of the study were used as a covariate, and weekly or biweekly means per cow were repeated measures. Interactions of treatment and parity ($P < 0.10$) were detected for fat and protein percentages. There were no differences in components for primiparous cows, whereas IB+MB increased ($P < 0.09$) protein percentage by 0.04 and 0.08 in multiparous cows compared with CON and MB treatments, respectively. IB+MB increased ($P < 0.01$) fat by 0.23 to 0.31% units compared with other treatments. Milk yield and DMI were not affected, but treatment interacted with week ($P = 0.06$) for ECM/DMI; IB+MB increased ($P < 0.10$) ECM/DMI by 0.15 (main effect means difference) compared with CON and MB, mainly during the earlier weeks of the study after which differences decreased as cows entered late lactation. The IB+MB treatment optimized feed efficiency in our study and not at the expense of BW gain. Further research is needed to optimize isoacids formulas under differing dietary conditions.

Key Words: branched-chain volatile fatty acids, feed efficiency, cellulolytic bacteria

T107 The effects of cut height and fungicide application on whole-plant corn silage yield. F. F. Cardoso*[1], S. E. Kemp[1], R. Schmidt[2], and F. C. Cardoso[1], [1]*University of Illinois*, *Urbana, IL*, [2]*Lallemand Animal Nutrition*, *Milwaukee, WI.*

Whole-plant corn silage (WPCS) is an indispensable forage in the diet of dairy cows due to its high-energy content. However, the amount of energy available to the cow depends on the quality of the WPCS. The objective of this study was to determine the effects of harvest cut height and foliar fungicide application on brown mid-rib (BMR) WPCS yield. Foliar fungicide (pyraclostrobin, C19H18ClN3O4 + metconazole, C17H22ClN3O; Headline AMP, BASF, Florham Park, NJ) treatments were randomly assigned to 1 of 24 0.21-ha plots. Treatments were assigned in a completely randomized block split-plot design as follows: control (CON) receiving no fungicide application and fungicide (FUN) receiving one application applied at the vegetative tassel stage (VT). Once corn reached growth stage R5, plants were collected, chopped, and tested for dry matter (DM) until all plots were at the 32–35% DM range. At a theoretical chop length of 2 cm [John Deere 8370 and a Dion F-41 Silage Chopper with kernel processor (1 mm)], corn was harvested 4 rows at a time at 2 separate cut heights low-cut (LC; 30.5 cm) and high-cut (HC; 56 cm). WPCS was collected right off the chopper as silage was being loaded into a horizontal silo bag. Dry matter of the WPCS was higher for FUN compared with CON (32.0% and 31.12%, respectively; $P = 0.02$). Gross yield of WPCS was lower for HC compared with LC (21,224 and 25,083 kg/ha, respectively; $P < 0.0001$) with no fungicide effect ($P = 0.67$). Dry matter yield of WPCS was lower for HC compared with LC (6,702 and 7,913 kg/ha; $P < 0.0001$) with no fungicide effect ($P = 0.25$). There was no interaction between height and FUN ($P > 0.15$). In conclusion, fungicide application had an effect on DM of WPCS and cut-height interfered with WPCS yield.

Key Words: whole-plant corn silage, fungicide, yield

T108 Effect of different levels of *Ascophyllum nodosum* meal on iodine metabolism in Jersey cows. M. Ghelichkhan*[2], L. H. P. Silva[2], R. C. R. Tinini[1], J. G. Dessbesell[1], M. A. Zambom[1], and A. F. Brito[2], [1]*Universidade Estadual do Oeste do Paraná*, *Marechal Cândido Rondon, Brazil*, [2]*University of New Hampshire*, *Durham, NH.*

Previous research showed that over 50% of organic dairies in the Northeast and upper Midwest US feed *Ascophyllum nodosum* meal (ASCO) known to be a rich source of iodine (I). Thus, ASCO supplementation is challenging because excess I intake may increase milk I above the 500-µg/L threshold recommended for consumers. We aimed to investigate the effects of different levels of ASCO on I metabolism in lactating dairy cows. Twenty organic-certified Jersey cows (102 ± 52 DIM) were paired by DIM or milk yield and assigned to treatments in a randomized complete block design. The experiment was conducted as follows: 400 g/d of ASCO was fed during 3 wk [elevated-phase; period 1 (P1)] then dropped to 100 g/d for another 3 wk (stepdown-phase; P2) and completely removed from the diet during the last 3 wk of the trial (withdraw-phase; P3). Cows were fed (DM basis) 60% alfalfa hay (19.6% CP, 43.6% NDF) and 40% concentrate (13.3% CP, 50% starch). Control (CON) cows received no ASCO. Milk, blood, and urine samples were collected on wk 3, 6, and 9. Additional milk samples were taken at 24, 48, 72, 96, and 168 h after ASCO supplementation was discontinued in the end of P2. Data were analyzed with repeated measures in SAS using MIXED procedure. Rate of milk I decline was estimated by regressing the natural log of milk I concentration on time of sampling using a nonlinear method for determining the slope in SAS. Diet by period interactions were observed for milk, plasma, and urine I concentrations, with cows fed ASCO showing greater values in P1 and P2 and no difference in P3 (Table 1). The slope of milk I decline over time was calculated as 0.76%/h according to the equation $Y = 6.26 (\pm 0.13) - 0.0076x (\pm 0.0013)$. Overall, feeding 100–400 g/d of ASCO elevated milk I above recommended levels for humans, and about 48 h are needed to bring milk I below 500 µg/L.

Key Words: iodine, organic, seaweed

T109 Phenotypic correlation of residual feed intake in mid and early lactation in Holstein cows. M. M. Nehme*, F. Peñagaricano, and J. E. P. Santos, *University of Florida*, *Gainesville, FL.*

Objectives were to establish phenotypic correlation between residual DMI (RFI) in mid lactation (ML, wk 9 to 13 postpartum) with RFI in early lactation (EL, wk 1 to 5 postpartum) in Holstein cows and determine associations between RFI in ML and performance in EL. Data from 155 primiparous and 245 multiparous enrolled in 4 experiments were collected. Cows were fed diets as TMR and amounts of DM offered and refused were measured daily from 1 to 91 DIM. Milk yield and BW were measured daily and milk sampled from 2 consecutive milkings twice a week was analyzed for fat, protein and lactose. Energy-corrected milk (ECM)

Table 1 (Abstr. T108). Iodine metabolism

	P1		P2		P3			*P*-value	
Item	CON	ASCO	CON	ASCO	CON	ASCO	SEM	Trt	Trt × P
Milk I, µg/L	198^{b}	854^{a}	265^{b}	578^{a}	186	218	57.0	<0.01	<0.01
Milk I, mg/d	4.89^{b}	20.50^{a}	6.44^{b}	14.13^{a}	4.51	5.24	1.70	<0.01	<0.01
Plasma I, µg/L	146^{b}	719^{a}	135^{b}	282^{a}	159	173	21.3	<0.01	<0.01
Urine I, µg/L	88.5^{b}	412^{a}	88.8^{a}	125^{a}	52.8	62.3	27.3	<0.01	<0.01

and net energy secreted (NES) in milk were calculated from yields of milk and milk components. Body condition was evaluated weekly. Metabolic BW (MBW) was calculated from $BW^{0.75}$. Body energy change (BEC) was calculated from daily change in BW and the BCS using NRC (2001) equation: BEC = [2.88 + (1.036 x BCS)] × BW change. Net energy balance (NEB) was calculated from energy intake and needs for maintenance and NES. Data were analyzed with the MIXED and CORR procedures of SAS. Intake of DM was regressed as a function of NES, MBW, BEC, parity, season of calving, and treatment within experiment. The error term in the model defined RFI. Cows were ranked by RFI in ML and categorized into quartiles as Q1 (most efficient) to Q4 (least efficient) (Table 1) Performance in EL was evaluated as a function of RFI quartile in ML. The phenotypic correlation between RFI in ML and EL was 0.43 (CI = 0.34–0.50). The correlation between BEC and NEB was 0.46 (CI = 0.38–0.53). Increasing efficiency in ML resulted in a linear decrease in DMI and NEB in EL; however, ECM yield and BEC were not affected by quartile of RFI. Re-ranking in phenotypic RFI exists between mid and early lactation, but selecting for RFI in ML does not seem to negatively affect cows in EL.

Table 1 (Abstr. T109). Quartile of RFI in mid lactation and performance in early lactation

Item	Q1	Q2	Q3	Q4	SE
RFI, kg/d*	−1.0	−0.2	0.3	0.9	0.2
DMI, kg/d*	16.0	16.6	17.5	18.3	0.3
ECM, kg/d	37.3	37.9	39.0	38.7	0.8
BEC, Mcal/d	−8.8	−9.0	−7.4	−7.8	0.7
NEB, Mcal/d*	−9.2	−8.2	−7.4	−6.1	0.5

*Linear ($P < 0.05$).

Key Words: residual feed intake, efficiency, phenotype

T110 Effects of acute intravenous trimethylamine *N*-oxide infusion on the bovine lipidome and metabolome during early lactation.

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Bacteria convert choline and carnitine to trimethylamine in the gastrointestinal tract. In the liver trimethylamine is converted to trimethylamine *N*-oxide (TMAO), which may contribute to metabolic disease in rodents. In an exploratory manner, our objective was to determine whether TMAO modulates metabolism in cows. Eight early lactation Holstein cows (30.4 ± 6.41 DIM) were enrolled in a study with a 4 × 4 replicated Latin square design. Cows were intravenously infused TMAO at 0 (control), 20, 40, or 60 g/d for 6 d. Washout periods lasted 9 d. Blood, milk, and liver tissue were collected on d 6. Untargeted lipidomics (nonpolar hydrophobic lipids; plasma) and metabolomics (polar hydrophilic compounds; serum, milk, and liver) were performed using C30 and pHILIC columns on a mass spectrometry platform in positive and negative modes, respectively. Statistical analysis was performed using MetaboAnalyst 4.0 following generalized log-transformation and auto-scaling. Data were analyzed using ANOVA and partial least squares discriminant analysis (PLS-DA). Following summation within lipid chain length, lipidomics analysis revealed 143 plasma lipids. The PLS-DA model distinguished TMAO treatments; however, only 44 lipids (~31%) were modified by TMAO treatment. No apparent pattern behaviors were observed. Examples of changes were limited to phosphatidylcholines (e.g., PC 36:4 and 37:2 were lower and PC 37:5 was higher in cows infused 60 g of TMAO/d, relative to control; false discover rate <0.05). Metabolomics analyses revealed 52 serum, 12 milk, and 39 liver compounds with a mzCloud mass spectral score > 75%; however, detected metabolites exceeded 100 for each sample type. These included amino acids, creatine, carnitine, and choline. We were not able to distinguish treatment metabolomes with PLS-DA. In addition, ANOVA did not detect differences in any metabolites with TMAO treatment. We conclude that the acute intravenous infusion of TMAO does not modify the bovine metabolome. Muted changes in the plasma lipidome in response to TMAO also suggest that this metabolite does not overtly modify metabolism in cows.

Key Words: cow, metabolome, trimethylamine *N*-oxide

T111 A dynamic approach for BCS prediction in NDS Professional.

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Based on the body reserves equation (NRCS 2001), calculated as energy inputs minus energy for maintenance, pregnancy, milk yield (MY) and growth, the CNCPS is able to predict BCS. One limitation of the model is that the MY is a set value for the breed therefore, especially when evaluating medium to low producing cows, the accuracy of the model may be reduced. The aim of this study was to validate, and possibly improve, the BCS prediction of the CNCPS by including "cows' current potential" in the equation. The "cows' current potential" was calculated as predicted peak milk yield over breed-specific peak; in the equation, the fat catabolism equation proposed by Johnson et al. (2016) has also been taken into account. Data regarding lactation number, parity, age and BW, BCS, milk yield and composition at 5 DIM from 63 pluriparous Jersey cows were included in the alternative model (M_{BCS}). The observed BCS taken at 35 DIM was used to validate the models. The observed values were regressed on the ones predicted by the CNCPS and M_{BCS} models and RMSPE was calculated. The significance of the deviation of the intercept from 0 and the slope from 1 was analyzed by *t*-test. Both R^2 and RMSPE indicated that the M_{BCS} performed better than the CNCPS model. The CNCPS model had R^2 lower than 0.60 with M_{BCS} having R^2 of 0.66. The CNCPS model had a slope of 0.2587 ($P < 0.05$) whereas the M_{BCS} had a slope of 0.6134. The CNCPS model consistently overestimated loss of BCS at 35 DIM with low-producing cows, whereas it was more accurate with high-producing animals. Although the R^2 is relatively low in both models, probably due to the limited size of the observed data set, the M_{BCS} better predicts BCS at 35 DIM for both high-producing and medium to low-producing cows. The results show that although the BCS prediction still needs improvement, the dynamic model proposed by NDS Professional has the advantage to better predict fat catabolism and, therefore, BCS especially when working with medium to low producing dairy cows. Thus, providing the users with more accurate decision-making tools. Both the M_{BCS} and the fat catabolism curve are now implemented in NDS Professional (RUM&N, Italy).

Key Words: modeling, CNCPS, BCS

T112 Effects of rumen-protected lysine fed to Holstein cows prepartum and the blood amino acids and metabolites profile on their calves.

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We aimed to determine the effects on dairy calves when their dams were fed rumen-protected lysine (RPL; AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL; 0.54%DM of TMR as top dress) 28 d before calving. Seventy-two Holstein calves; (male, n = 37 and female, n = 35), blocked based on dams blocking criteria, were assigned 2 different dietary treatments based on dams prepartum treatment of RPL supplementation (L) or without (C). At the time of birth (0.5 ± 2h after), before colostrum was given, blood samples were collected into separation blood tubes (Becton Dickinson, Rutherford, NJ). Plasma amino acid concentrations were determined by HPLC (Ajinomoto's diagnostic lab, Japan). Fecal score was measured weekly and health status were evaluated daily. Statistical analysis was performed using GLIMMIX and MIXED procedures of SAS. Male calves in C had higher ($P = 0.05$) medicated events compared with male calves in L. Thus, male calves in C also showed higher ($P = 0.03$) antibiotic utilization compared with male calves in L. Plasma lysine concentrations were not different for calves in C or L ($P = 0.26$; 10.09 µmol/dL vs 10.5 µmol/dL respectively). Plasma methionine concentrations were not different ($P = 0.95$) among treatments (L = 1.194 µmol/dL and C = 1.198 µmol/dL). Male calves tended to have higher ($P = 0.10$) plasma methionine concentrations (1.24 µmol/dL) than female calves (1.15 µmol/dL). There was an interaction of treatment by sex for plasma glutamic acid concentrations ($P = 0.007$); where females in C (5.3 µmol/dL) had higher ($P = 0.04$) concentrations than males in C (3.9 µmol/dL); and males in C tended ($P = 0.06$) to have lower concentrations than males in L (5.1 µmol/dL). Another interaction of treatment by sex for plasma alanine concentration ($P = 0.05$); where males in C (0.44 µmol/dL) tended to have lower ($P = 0.06$) concentrations than males in L (0.71 µmol/dL). In conclusion, feeding cows with RPL prepartum reduced medicated events in their male offspring. Additionally, male calves from cows supplemented with RPL tended to have increased plasma alanine and glutamic acid concentrations than calves from cows not treated with RPL.

Key Words: calf, amino acids, health

T113 Effects of rumen-protected lysine fed to Holstein cows prepartum and the outcome on their calves. B. L. Thomas*[1], A. R. Guadagnin[1], L. K. Fehlberg[1], Y. Sugimoto[2], I. Shinzato[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana-Champaign, IL*, [2]*Ajinomoto Co. Inc, Tokyo, Japan*.

This experiment was conducted to determine the effects on dairy calves when their dams were fed rumen-protected lysine (RPL; AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL; 0.54%DM of TMR as top dress) 28 d before calving. Seventy-two Holstein calves; (male, n = 37; and female, n = 35), blocked based on dams blocking criteria, were assigned into 2 different dietary treatments also based on dams prepartum treatment of RPL supplementation (L) or without (C). Birth weight was measured at 1 ± 3h of birth, before colostrum administration. Calves were given 3.8L of colostrum replacer (Land O'Lakes Bovine IgG Colostrum Replacer, Land O'Lakes, Inc., Arden Hills, Minnesota, USA). All calves were placed in individual hutches (calf-tel, ample Corp., Germantown, WI). Calves were in the hutches for 56 d and are given water ad libitum. Calves were then given milk replacer (Advanced Excelerate, Milk Specialties, Carpentersville, IL; 28.5% CP, 15% fat) twice daily until 42 d of age. Grain starter (19.9% CP, 13.5% NDF, Ampli-Calf Starter, Purina, St. Louis, MO) was given from d 1 of age until d 56 at ad libitum. Calves BW, body length, heart girth, wither height, hip width, and hip heights were measured weekly, on weaning day (d 42), and end of trial (d 56). Statistical analysis was performed using the MIXED procedure of SAS. During wk 1 to 6 (wet period) male calves (47.01 kg) had greater ($P < 0.0001$) initial BW than females (41.49 kg). From wk 1 to 8, male calves had greater ($P = 0.002$) final BW than females (86.91 kg vs 78.96 kg, respectively). Male calves in the wet period (7.59 kg/kg; $P < 0.0001$), and wk 1 to 8 (7.00 kg/kg; $P < 0.0001$) had a greater gain: feed ratio than females (6.77 kg/kg and 6.3 kg/kg, respectively). Calves in C (0.08 cm/d) had higher ($P = 0.03$) ADG for heart girth; than calves in L (0.06 cm/d). Calves in L tended to consume more DMI ($P = 0.06$; 813.89 g/d) and CP ($P = 0.07$; 234.47 g/d) than those in C (792 g/d and 228.42 g/d, respectively). In conclusion, calves from dams fed RPL prepartum had improved overall growth and intake than calves from dams not receiving RPL.

Key Words: calf, lysine, average daily gain

T114 Effect of essential oils and amylase supplementation on productive performance of lactating dairy cows. A. Silva[1], C. S. Cortinhas*[2], T. S. Acedo[2], M. J. F. Morenz[3], F. C. F. Lopes[3], M. B. Arrigoni[1], M. H. Ferreira[4], and T. L. Jaguaribe[4], [1]*São Paulo State University, Botucatu, SP, Brazil*, [2]*DSM Nutritional Products, São Paulo, SP, Brazil*, [3]*Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil*, [4]*Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil*.

The objective with this study was to evaluate the effects of essential oils supplementation, in combination or not with amylase, on productive performance of lactating dairy cows. Thirty-six Holstein cows were blocked by days in milk (78.0 ± 9.5) and allocated according the milk production into the treatments: a) control (CTRL), no additives; b) essential oils (EO), addition of a blend of essential oils (100 mg/kg of DM; CRINA Ruminants, DSM Nutritional Products, Basel, Switzerland); and c) essential oils + amylase (EOam), combination of the essential oils (100 mg/kg of DM) and α-amylase (625 mg/kg of DM, Ronozyme RumiStar, DSM Nutritional Products, Basel, Switzerland). Basal diets were formulated to contain 291 g/kg of NDF, 166 g/kg of CP and 304.4 g/kg of starch (DM basis). Animals were adapted to diets for 14 d, and then evaluations were carried out for 60 d. Cows were milked twice daily, and the production recorded automatically by the software Delpro Manager System. Milk samples were collected thrice weekly in 2 sequential milkings, to determine the content of fat, protein, lactose and urea nitrogen in milk. Data were analyzed using the mixed procedure of SAS. Differences among treatments were analyzed through the following contrasts: C1 = control versus feed additives; C2 = EO versus EOam. There was no difference for dry matter intake (23.8 kg/day, $P = 0.44$). Feed additives (EO and EOam) increased milk yield ($P = 0.03$) in comparison to the CTRL (31.8 vs 29.1, respectively). Means values did not differ between diets containing EOam and EO (32.1 vs. 31.5 kg/day; $P = 0.57$). However, higher feed efficiency was obtained in diet containing EOam versus EO (1.43 vs 1.24, respectively; $P = 0.02$). There was no differences ($P > 0.05$) for the contents of milk fat (37.7 g/kg), milk protein (30.2 g/kg), lactose (45.9 g/kg) and urea nitrogen (17.2 mg/dL). Also, no response was observed for fat-corrected milk (32.3 kg/day) and energy-corrected milk (31.6 kg/day). The use of essential oils associated with amylase increases the milk yield and feed efficiency in dairy cows.

Key Words: additives, feed efficiency, milk yield

T115 Effects of dietary 25-hydroxyvitamin D_3 for prepartum dairy cows receiving acidogenic diet. A. Silva[1], C. S. Cortinhas*[2], T. S. Acedo[2], M. J. F. Morenz[3], F. C. F. Lopes[3], M. B. Arrigoni[1], M. H. Ferreira[4], and T. L. Jaguaribe[4], [1]*São Paulo State University, Botucatu, SP, Brazil*, [2]*DSM Nutritional Products, São Paulo, SP, Brazil*, [3]*Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil*, [4]*Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil*.

The aim with this study was to evaluate the effects of 25-hydroxyvitamin D_3 or cholecalciferol supplementation on milk yield, milk composition and plasma concentration of minerals. Forty multiparous Holstein dairy cows were blocked by parity at 30 d of the expected day of calving to receive an acidogenic diet (365 g/kg of NDF and 146 g/kg of CP, DM basis; −108 mEq/kg) with the treatments mixed in the TMR: a) control, cholecalciferol (Rovimix D_3 500, DSM Nutritional Products, Basel, Switzerland) at 2.500 UI/kg of DM; b) HyD: 25-OH-D_3 at 0,3 mg / kg of DM (Rovimix HyD, DSM Nutritional Products, Basel, Switzerland). After calving, cows were fed with the same diet. Colostrum yield was evaluated at 6 h after calving. Cows were milked twice daily and the production recorded during the first 50 d of lactation. Milk samples were collected thrice weekly in 2 sequential milkings to determine the content of fat, protein, lactose and urea nitrogen in milk. Blood samples were collected from coccygeal vein on days −7, 0, 1, 2 and 21 relative to parturition for determination of total Ca (tCa), ionized Ca (iCa), and total P (tP); and on days −7, 0 and 21 for 25-hydroxyvitamin D_3 analysis. Data were analyzed using the mixed procedure of SAS. Statistical significance was considered at $P \leq 0.05$ and the trend at $0.05 < P \leq 0.10$. HyD supplementation increased milk yield (32.5 vs. 29.9 kg/day; $P = 0.04$), fat-corrected milk yield (33.0 vs. 30.0 kg/day; $P = 0.03$), energy-corrected milk (32.4 vs. 29.5 kg/day; $P = 0.03$), and protein yield (0.97 vs. 0.889 kg/day; $P = 0.04$) compared with control group. Also, HyD tended to increase fat yield (1.17

vs. 1.05 kg/day; $P = 0.06$), colostrum yield (7.40 vs 5.63 kg; $P = 0.07$), and DM intake on prepartum (11.73 vs. 10.70 kg/d; $P = 0.08$). There was no difference ($P > 0.10$) for DM intake postpartum, and concentrations of fat, protein, lactose and urea nitrogen. In addition, HyD increased plasma concentration of 25-hydroxyvitamin D_3, but no effect was observed on iCa, tCa, tP ($P > 0.10$). The HyD supplementation on prepartum dairy cows increase milk yield and improve composition in early lactation.

Key Words: 25-hydroxyvitamin D_3, cholecalciferol

T116 A meta-analysis to compare feed and milk N efficiency of Holstein and Jersey cows.

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A meta-analysis was conducted to compare feed efficiency (FE) and milk N efficiency (MNE) between Holstein (HO) and Jersey (JE) cows. The data set was developed using the Web of Science database with the literature search ranging from 1980 to December 2019. The terms "Holstein," "Jersey," "milk yield," and "milk production" were used in the Web of Science search engine to identify peer-reviewed articles yielding 166 hits. From this initial pool, 66 papers containing both breeds were selected with 12 articles retained in the final data set. The following criteria were adopted to include studies in the meta-analysis: (1) both purebred HO and JE used in the same trial; (2) DMI/breed or individual treatments; and (3) milk yield and composition. Feed efficiency was calculated as milk yield/DMI and ECM yield/DMI, while MNE was computed as milk N yield/N intake. Crude protein intake was estimated by multiplying DMI (kg/d) by the respective treatment CP concentration and converted to N intake (g/d). Milk N was computed by dividing milk protein by 6.38 for standardizing MNE. Breeds DIM averaged (mean ± SD) 118 ± 56, and mean BW for HO and JE was 616 ± 55.4 and 437 ± 56.2, respectively. Sixty-five observations (treatment means) were used in the meta-analysis, with 1 observation excluded from the data set as it was deemed outlier based on the −2.5 to 2.5 range of studentized residual. Data were analyzed using the MIXED procedure of SAS with the fixed effect of breed and the random effects of study and the breed by study interaction included in the statistical model. Holstein had greater DMI (20.9 vs. 17.2 kg/d; $P < 0.001$) and milk yield (31.2 vs. 22.3 kg/d; $P < 0.001$) than JE. Likewise, ECM yield was 16% greater in HO compared with JE (33.4 vs. 28.8 kg/d; $P < 0.001$). Holstein was also more efficient than JE to convert DMI into milk yield (1.51 vs 1.31; $P < 0.001$). However, when FE was calculated as ECM/DMI, JE had greater FE than HO (1.70 vs. 1.60; $P = 0.03$). In contrast, MNE did not differ significantly between breeds (mean = 29%). In brief, our meta-analysis revealed that HO and JE differ in FE but with similar MNE.

Key Words: efficiency, dairy cow, nitrogen

T117 Energy partition in Holstein × Gyr lactating cows raised under tropical conditions.

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It is believed that in tropical conditions crossbred Holstein × zebu cows has a difference in the energy partition of the diet when fed restricted or ad libitum. Therefore, the objective of the study was to evaluate the effects of nutrition plans and parity on the energy partition. Twenty-eight late lactation crossbred (3/4 Holstein × 1/4 Gyr) cows (BW = 552 ± 88.13 kg and MY = 15.12 ± 4.16 kg/d), were evaluated using a respiromimetric chamber under tropical climate conditions. Two nutrition plans (ad libitum and restriction) and 2 parities (primiparous and multiparous), were used in a 2 × 2 factorial arrangement of treatments in a completely randomized design. The cows were fed a diet consisting of 74% corn silage and 26% concentrate and restricted cows were fed 80% of the required net energies for multiparous cows and 85% for primiparous cows. There were no effects of parities in energy partition. Cows fed ad libitum had higher intakes of gross energy (306 vs. 179 MJ/d), digestible energy (212 vs. 121 MJ/d), metabolizable energy (185 vs. 104 MJ/d) and net energy for lactation (65 vs. 33 MJ/d); showed highest heat production (102 vs. 75 MJ/d), energy loss as feces (94 vs. 57 MJ/d), urine (12 vs. 8.5 MJ/d) and methane (14. vs. 9.0 MJ/d), compared with restricted cows ($P < 0.001$). There was interaction for heat production between feeding levels and parities ($P = 0.002$), with higher values for primiparous cows fed ad libitum. The metabolizable energy efficiency used for maintenance was 0.61 and 0.58 MJ ($P = 0.016$) for ad libitum and restricted treatments. The energy partitioning for body tissues was higher for ad libitum cows (0.34 vs. 0.32 MJ/MJ, $P < 0.001$). However, cows fed restriction diet had lower metabolizable fraction use for heat production (0.54 vs. 0.73 MJ/MJ, $P < 0.001$). The energy balance (Mcal/d) was 5.7 for ad libitum cows and −0.1 for restricted cows ($P < 0.001$) owing to restriction level imposed to multiparous cows. Ad libitum cows had higher energy balance, in response to higher levels metabolizable energy, above maintenance exigencies.

Key Words: calorimetry, Girolando, metabolism

T118 The effect of a mycotoxin deactivator on the vitamin and mineral status of lactating dairy cows.

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When using a mycotoxin deactivator product (MDP) one possible downfall could be the unspecific and undesired binding of vitamins and minerals. The objective of these 2 trials was to evaluate the in vivo effect of application of an MDP product on blood levels of vitamins and minerals in lactating dairy cows. For the first trial (trial 1), 12 Holstein cows in mid-lactation were used in a 3x3 4 times replicated Latin square design. Cows received either an uncontaminated TMR (CTR), a TMR contaminated with *Fusarium* mycotoxins (MTX), or the contaminated TMR supplemented with an MDP (35 g/animal/day) (MDP). Each of the experimental periods consisted of a 3-week treatment period followed by a 2-week clearance period. For the second trial (trial 2), a total of 20 Simmental cows in mid-lactation were assigned to either a control group (CTR) or a treatment group (MDP) receiving 60 g/head/day of the same MDP as in trial 1 for 7 d. In either trial, blood samples were taken from each animal before the administration of the product, during and/or at the end of the product application (D0, D14 and D21 in trial 1 and D0 and D7 in trial 2). Blood samples were analyzed for calcium, chloride, magnesium, phosphate, potassium, sodium and zinc in both trials 1 and 2 and for vitamins A, D3, B1, B2, B6 and B12, as well as copper, iron, manganese and selenium in trial 2. In trial 1, data were analyzed as repeated measurements using the MIXED procedure of SAS 9.3. The data of trial 2 were analyzed using independent-samples *t*-test in SPSS 22.0. We did not detect a significant difference between treatments for any measured parameters in either trial. For example, the levels of manganese or vitamin B12 did not differ significantly between treatment groups in trial 2 at D7 (Manganese: CTR: 3.76 ± 1.46 ng/L vs. MDP 4.17 ± 0.81 ng/L $P = 0.63$, Vitamin B12: 1CTR 29.05 ± 40.70 μg/L vs. MDP 159.41 ± 37.54 μg/L, $P = 0.56$). In conclusion, the administration of this specific MDP did not affect the levels of vitamins or minerals in the blood of dairy cows suggesting that at this inclusion levels no undesired binding took place.

Key Words: mycotoxin deactivator product, vitamins, minerals

Ruminant Nutrition: Carbohydrates and Lipids

T119 Ruminal degradability and bypass fraction of a coated omega-3 source. L. R. Royo*[1], T. de Evans[2], M. Puyalto[1], J. J. Mallo[1], and M. D. Carro[2], [1]*Norel SA, Madrid, Spain*, [2]*Dpto. Producción Agraria, ETSIAAB, Universidad Politécnica de Madrid, Madrid, Spain.*

The aim of this study was to analyze ruminal degradability and bypass fraction of a C18:3 source (linseed oil), either free or coated with palm stearin. Two samples (free linseed oil with palm stearin (LSS) and coated linseed oil, commercialized as HiFlax (HFX) by NOREL) were evaluated in an in vitro trial. Ruminal content was obtained from 3 cannulated sheep fed a 50:50 concentrate:forage ration, and filtered through gauze to get the fluid. Intact samples (80 mg of HFX or a mixture of 25 mg linseed oil and 55 mg palm estearin) were weighed in vials containing 1 g of the diet fed to animals. Vials were filled up with 80 mL of a 1:4 ruminal fluid:incubation medium mixture, and incubated at 39°C for 12 h (passage rate 8%/h). Each sample was incubated with the rumen liquid of each sheep to obtain 3 replicates. After incubation, vials contents were placed in weighed sterile plastic containers that were immediately frozen at −80°C to stop fatty acids (FA) biohydrogenation, and lyophilized. The fat content and FA profile of the samples and incubation residues were analyzed. The disappearance of each C:18 FA was calculated as the difference between the amount added in the vials with the ruminal inoculum, basal substrate and sample, and the amount in the incubation residue. The bypass fraction of each FA was calculated as 100 minus the measured disappearance. Data were analyzed using the PROC MIXED of SAS, where the fixed factor was the sample and the random factor was the inoculum. The C18:3 in LSS was extensively biohydrogenated (90.7%), but the C18:3 in HFX showed lower disappearance ($P = 0.093$; 61.3%) and therefore a greater bypass fraction (38.7%). There was no difference ($P > 0.05$) in the disappearance of C18:2 and C18:1 between the 2 samples (53.2 and 62.9% for C18:2, and 10.0 and −34.8% for C18:1; values for HFX and LSS, respectively). For LSS, the amount of C18.1 in the incubation residue was greater than that incubated, as a result of more extensive ruminal biohydrogenation of C18:3. It is concluded that the use of palm stearin to coated linseed oil utilized in HFX is a valid strategy to protect C18:3 from ruminal biohydrogenation.

Key Words: C18:3, rumen, biohydrogenation

T120 Assessing fatty acids and starch solubility in corn grain and corn silage using in vitro method. X. Huang*[1], J. de Souza[2], and R. Ward[1], [1]*Cumberland Valley Analytical Services*, *Waynesboro, PA*, [2]*Perdue AgriBusiness LLC*, *Salisbury, MD.*

Fatty acids and starch are important sources of energy in the diet, but they may also interact and change ruminal biohydrogenation intermediates that may affect milk fat synthesis. When fatty acids and starch are released fast, the production of biohydrogenation intermediates linked to milk fat depression may increase since unesterified fatty acids cannot be completely biohydrogenated to stearic acid. The solubility of fatty acid and starch is assumed to be associated with their degradation rates in rumen. The objective of this study was to assess fatty acid and starch solubility in corn grain and corn silage and their potential correlation. Twenty-seven corn grain samples [17 high-moisture corn samples (HMC) and 10 snaplage samples] and 57 corn silage samples (23 BMR samples) were collected and sent to CVAS (Waynesboro, PA) for in vitro fatty acid and starch solubility analysis. The soluble fatty acid in corn grain and corn silage were 25 ± 16 and 25 ± 14% of total fatty acids respectively. The starch solubility of corn grain and corn silage were 43 ± 22 and 62 ± 18% of total starch respectively with significant difference between HMC and snaplage (33 ± 17% vs 62 ± 15%, $P < 0.05$). The soluble fatty acids proportion was positively correlated with soluble starch proportion (0.84) and soluble dry matter in HMC (0.91) but not in snaplage (0.33 and 0.34). The soluble fatty acids proportion was also positively correlated with soluble starch proportion (0.64) and soluble dry matter in corn silage (0.62) without difference between traditional corn silage and BMR. The fatty acids and starch content are positively correlated in corn silage (0.73) but not correlated in corn grain. Overall, the solubility of fatty acid and starch in corn silage and HMC are similar. The fatty acids solubility was likely associated with starch and dry matter solubility, which indicated that this method may be useful to rank feed ingredients according to the soluble starch and fatty acid levels. Consideration for this correlation present maybe warranted for improving our ability to predict ruminal fermentation and biohydrogenation under different diets.

Key Words: fatty acids, starch, solubility

T121 Dietary energy source and rumen-protected amino acids: Effects on CH_4 emissions and heat production in lactating dairy cows. Y. Zang*[1], L. H. P. Silva[1], Y. Geng[2], M. J. Lange[3], N. Q. Dattolico[1], N. L. Whitehouse[1], M. Miura[4], M. A. Zambom[3], and A. F. Brito[1], [1]*University of New Hampshire*, *Durham, NH*, [2]*Chinese Academy of Agricultural Sciences*, *Beijing, China*, [3]*Universidade Estadual do Oeste do Paraná*, *Marechal Cândido Rondon, Brazil*, [4]*Ajinomoto Co. Inc*, *Kawasaki-shi, Japan*.

Our previous research showed that reduced dietary starch level increased CH_4 energy losses and tended to decrease milk energy of dairy cows receiving MP-deficient diets. Supplementing rumen protected (RP)-fat to reduced starch diets may improve energy use efficiency in dairy cows. Therefore, our objective was to examine the effects of dietary energy source (starch vs. RP-fat) and RP Met, Lys, and His (MLH) on enteric CH_4 emission and heat production (HP) in dairy cows fed MP-deficient diets. Sixteen multiparous Holsteins cows (112 ± 28 DIM) were used in a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments. Each period lasted 21 d with 14 d for diet adaptation and 7 d for sample collection. Treatments were high starch (HS), HS + RPMLH, reduced starch + RP-fat (RSF), and RSF + RPMLH. Basal diets consisted of (DM basis) 50% forage and 50% concentrate. The HS diet contained 26% ground corn, while the RSF diet had 16% ground corn replaced with 15% soyhulls and 1.5% RP-fat (i.e., palmitic acid-enriched supplement). Dietary NE_L, starch, and CP averaged 1.53 Mcal/kg, 32.6% and 15.9% for HS diets, and 1.59 Mcal/kg, 21.7% and 16.8% for RSF diets, respectively. Smartamine M, Aji-Pro L, and an Ajinomoto prototype RP-His product were fed to meet digestible MLH requirements. The GreenFeed system was used to measure gaseous fluxes, with HP estimated using the Bayat et al. (2019) equation: $HP (MJ/d) = 0.0184 \times QCO_2 (L/d) + 7.50$. Data were analyzed with the MIXED procedure of SAS. Dietary treatments had no effects on CH_4 production (mean = 534 g/d), CH_4 yield (mean = 18.5 g/kg of DMI), and CH_4 intensity (mean = 11.6 g/kg of ECM). Consequently, cows fed HS and RSF diets had similar CH_4 energy losses (7.03 vs. 7.12 Mcal/d). Moreover, emission of CO_2 (mean = 16.2 kg/d) and HP (mean = 37.8 Mcal/d) did not differ significantly across diets. No effect of RP-MLH supplementation on energy utilization was observed. In brief, our data suggest that ground corn can be partially replaced with soyhulls and RP-fat without changing energy use efficiency in lactating dairy cows.

Key Words: amino acid, dairy cow, energy source

T122 Evaluating rumen degradation of protected gelatin capsules filled with fish oil when fed to Holstein or Jersey lactating cows. O. M. Peña*, S. Saunier, K. Murphy, N. M. Long, G. L. Lascano, M. J. Aguerre, and T. C. Jenkins, *Department of Animal and Veterinary Sciences, Clemson University*, *Clemson, SC.*

The main objective of this study was to evaluate the effect of breed on consumption and ruminal degradation of fish oil capsules. Other objective was to evaluate the effect of capsule treatment on milk fatty acid composition and content. Three Holstein (H; mean ± SD; 154.6 ± 17.6 kg/d; DIM), and 3 Jersey (J; mean ± SD; 119.6 ± 22.5 kg/d; DIM) ruminally-fis-

Table 1 (Abstr. T123). Fermentation kinetics for corn at different maturities across storage methods

Item	ED	HM	TQ	BL	SE	*P*-value
MaxGas, mL/g of DM	337^{a}	316^{b}	305^{b}	291^{c}	3.75	<0.0001
kd, %/h	6.22^{a}	4.67^{b}	4.41^{bc}	3.82^{c}	0.223	<0.0001
Lag, h	8.88	8.92	8.15	8.44	0.377	0.50
DM, %	51.0	57.0	62.4	66.0	ND[1]	ND
Starch, % of DM	73.1	74.0	73.3	71.8	ND	ND
IVSD8h, % of Starch	43.8	34.9	35.1	34.8	ND	ND

[1]Not determined, single measurements.

tulated cows were randomly assigned to 1 of 3 treatments: (1) no capsules (CON), (2) untreated capsules (UC), and (3) treated capsules (TC) in a 3 × 3 Latin square design with 21-d periods. Cows were fed ad libitum a TMR composed of 10% alfalfa hay, 40% corn silage, 50% premix (DM basis) that contained 15.9% CP, 33% NDF and 27.6% starch. Samples and data collection were taken during the last 5 d of each period. Changes in weight and hardness (resistance to deformation) of TC were measured after 3 h of being in water at 39°C. Data were analyzed using JMP Pro 12 with the following preplanned orthogonal contrasts: (1) CON vs. (UC+TC), (2) UC vs. TG. There was no treatment effect on DMI (20.2 ± 1.18 kg/d). However, H consumed more than J (23.2 vs.17.3 kg/d; $P < 0.02$). Cows receiving UC and TC had higher ($P < 0.01$) milk yield compared with CON (30.7 vs 27.0 kg/d). Milk yield was higher ($P < 0.04$) for H vs J (34.6 vs. 24.4 kg/d). Milk fat % was not affected by treatments (mean ± SD 4.42 ± 0.39kg/d) but was numerically higher ($P < 0.17$) in J compared with H (4.90 vs 3.94%). Compared with control, feeding UC and TC increased the ruminal concentration of 20:5n-3 (0.033 vs. 0.064%; $P < 0.01$) and 22:6n-3 (0.029 vs. 0.074%; $P < 0.07$). In addition, J showed higher concentration of 20:5n-3 than H (0.061 vs. 0.047%; $P < 0.03$). It was observed that very few intact capsules were recovered from the rumen for both breeds. However, we were able to find several pieces of TC. Lab studies indicated that weight increased 60% and hardness was reduced by 98% post water incubation. Results from this study indicate that there was no effect of breed in capsule consumption. Data suggest that capsules were susceptible to breakage from abrasion when wet, both in the TMR and in the rumen.

Key Words: Holstein, Jersey, milk fat

T123 Effects of maturity and storage method on gas production kinetics of corn grains.

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The zein matrix forms during maturity of corn kernels and reduces starch digestibility by limiting microbial access to the starch. Drying and grinding for laboratory analyses may further confound factors affecting starch digestibility. The objective was to determine the effect of storage method of corn grain on fermentability at different stages of maturity. Ears from 97d corn were collected to obtain 4 maturities(M): early dent (ED), half milk line (HM), 3 quarter milk line (TQ), and black layer (BL). Kernels were stored (S) as refrigerated (R), dried for 4 h (D4), 8 h (D8), 16 h (D16) or frozen and thawed (FT) for analysis. A subsample of D16 was ground to 4mm to determine 8-h in vitro starch digestibility (IVSD8). Kernels (0.87 ± 0.14g DM) were cut in half and fermented with 60 mL Goering and Van Soest (1970) medium and 20 mL blended ruminal fluid from 2 Jersey steers (Diet = 13.1% CP and 31.3% starch as DM). Ankom RF modules were used to measure gas production (GP) for 120h during 3 runs. The model: $Y = \mu + \text{Run} + M + S + M \times S + \text{Error}$ was tested using aov() in R. Run interactions were nonsignificant. Interactions of MxS were significant, but minor in magnitude. Maximum gas production (MaxGas) and rate (kd) decreased with maturity ($P < 0.0001$) while there was no effect on lag (Table 1). Correlations between means of %DM and MaxGas, kd, and lag were −0.99, −0.96 and −0.76, respectively, across M. For MaxGas, R was greater than FT. For kd, R was greater than D4 and D8. For lag, R was greater than D16. Difference between ED and BL was 67, 60, 23, 37 and 50 mL/g DM, respectively, for R, D4, D8, D16 and FT. Results suggest maturity/DM and storage conditions can affect GP, and that measuring GP kinetics of half kernels can detect differences due to maturity or storage of starch in corn grains.

Key Words: starch, digestion, gas production

T124 Effect of increasing levels of roasted high oleic soybean on milk fat yield in lactating dairy cows.

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Oilseeds are an economical source of dietary fat, but the rumen available unsaturated fat they contain is a risk factor for diet-induced milk fat depression (MFD). However, high oleic soybeans have been shown to decrease risk for MFD compared with conventional soybeans. Additionally, it has been demonstrated that milk fat can be increased by increasing dietary fat in some situations. The hypothesis of this experiment was that feeding roasted high-oleic soybeans at an increasing rate would increase milk fat yield in high producing dairy cows. The objective was to demonstrate that high oleic soybeans have a low risk for milk fat depression and are an inexpensive source of dietary fat. Eight primiparous and 8 multiparous lactating Holsteins were arranged in a replicated 4x4 Latin square design with 21 d periods. Treatments were 0, 5, 10, and 15% of roasted high oleic soybeans (Plenish, Pioneer Inc. Johnston, IA). Roasted soybeans were substituted for soybean hulls and heat treated and solvent extracted soybean meal. Data were analyzed using the random effect of cow and period and fixed effect of treatment, parity, and their interaction. Preplanned contrasts tested the linear and quadratic effect of increasing roasted soybeans levels. There were no interactions of treatment and parity. Milk yield was not changed by treatments (mean 36.6 and 46.7 kg/d in primiparous and multiparous, respectively). Milk fat percent (4.07 to 4.17%) and yield (mean 1700 g) were also not modified by treatment. Milk protein percent was decreased from 3.07 to 2.92% ($P < 0.001$) and MUN was decreased from 15.2 to 11.9 mg/dL ($P < 0.001$) with increasing level of roasted high oleic soybeans, but milk protein yield was not changed. This may be due to differences in bypass protein or protein digestibility. In conclusion, roasted high oleic soybeans maintained milk and milk component yield when fed at up to 15% of the diet without induction of MFD.

Key Words: high oleic soybeans, milk fat depression, milk fat

T125 Supplementation of methionine and methionine analogs to diets with risk of biohydrogenation-induced milk fat depression.

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We have previously observed that the rumen available methionine analog 2-hydroxy-4-(methylthio)butanoate (HMTBa) reduced BH-induced MFD when feeding high unsaturated FA and high starch diets. The effect of other sources of methionine have not been investigated, but may play a role through both ruminal and post-ruminal mechanisms. Ten primiparous (97 ± 27 DIM; mean ± SD) and 26 multiparous (97 ± 33 DIM; mean parity = 3) cows were arranged in a 5 × 5 Latin square design. Treatments were (cow/d basis): unsupplemented control; 30 g/d of HMTBa (Alimet; Novus International, Inc., St. Charles, MO); 43 g/d of HMBi (MetaSmart, Adisseo, Alpharetta, GA); 12 g of HMTBa and 42.9 g of HMBi; and 26.5 g of rumen protected methionine (RPM; Smartamine M, Adisseo). Each period in the replicated design included low (d 1 to 20), moderate (d 21 to 27), and high (d 28 to 30) risk of BH-induced MFD phases. Risk for altered BH was increased by reducing diet NDF and increasing UFA and starch content. Data was analyzed using repeated measures in Proc Mixed. The model included the random effect of cow and period and the fixed effect of treatment and day. Subject was cow × period and the TOEP covariance structure was used. Overall milk fat concentration decreased from 3.83 to a 3.64% and trans-10 C18:1 increased from 0.45 to 1.23% of FA between the low- and the high-risk phases demonstrating moderate induction of BH-induced MFD. There was no interaction of treatment by dietary risk for milk fat concentration and yield, but there was an overall effect of treatment as both were increased by all treatments but HMBi from 0.11 to 0.16 percentage units and 85 to 132 g/d ($P < 0.05$). However, there was no effect of treatment on milk trans-10 C18:1. There was a treatment by day interaction for milk protein concentration and yield ($P < 0.05$) with RPM increasing milk protein concentration and yield most consistently. In conclusion, both rumen available HMTBa and rumen protected methionine decreased risk of MFD when increasing dietary unsaturated FA and starch.

Key Words: 2-hydroxy-4-(methylthio)butanoate (HMTBa), methionine, milk fat depression

T126 Effects of dietary deoiled lecithin supplementation on apparent fatty acid digestibility and absorption in dairy cows.

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Our objective was to evaluate the effects of dietary palmitic acid (PA) and lecithin on fatty acid (FA) digestion in cows. In a split-plot Latin square design, 16 Holstein cows (160 ± 7 DIM) were allocated to a main plot receiving a corn silage and alfalfa haylage-based diet with palm fat containing either moderate or high PA content at 1.75% of ration DM (MPA and HPA, respectively; BergaFat F-100 Classic or F-100 HP containing 87 or 98% PA, respectively; Berg + Schmidt, Hamburg, Germany; n = 8/group). On each palm fat diet, deoiled soy lecithin was top-dressed at 0, 0.12, 0.24, or 0.36% of ration DM in a replicated 4 × 4 Latin Square design. Following a covariate period, lecithin feeding spanned 14 d with feed and feces collected during the final 3 d of each experimental period. Feed and fecal FA were analyzed by GC-FID. Indigestible NDF was used as an internal marker to estimate fecal output and apparent FA digestibility. The statistical model included the fixed effects of PA supplement, lecithin dose, period, and their interactions as well as the random effect of cow. HPA increased 16C FA intake, relative to MPA ($P = 0.02$). Total and 16C FA digestibility were higher with MPA, relative to HPA (74 vs. 61% and 73 vs. 46%, respectively; $P < 0.01$); however, 18C FA digestibility was not modified by PA supplement. Total and 16C FA absorption were higher with MPA, relative to HPA ($P = 0.01$ and $P = 0.09$, respectively); however, 18C FA absorption was not modified by PA supplement. Lecithin dose linearly increased 18C FA intake in cows fed HPA (palm fat × lecithin, $P = 0.07$). Lecithin feeding did not modify total or 16C FA digestibility. Lecithin tended to cubicly affect 18C FA digestibility in cows fed MPA (palm fat × lecithin, $P = 0.06$). The absorption of total or 16C FA was not modified by lecithin; however, lecithin dose tended to linearly increase total FA absorption in cows fed HPA (palm fat × lecithin, $P = 0.15$). We conclude that increased dietary PA decreases FA digestibility and feeding unprotected deoiled soy lecithin does not modulate total or 16C FA digestibility in lactating cows.

Key Words: fatty acid digestibility, lecithin, palmitic acid

T127 Impact of dry ground corn particle size on production performance of dairy cows.

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The objective of this trial was to evaluate the impact of dry ground corn mean particle size on production performance of lactating cows. Fifty Holstein dairy cows were separated in 2 groups receiving diets with either coarsely (915 µm) or finely (660 µm) ground corn. Cows were housed in individual tie-stalls in the same barn, equipped with wooden side panels. The ground corn inclusion in the experimental diet was modest; only 3.1 kg DM/d or 14.5% of total diet DM, and the estimated total dietary starch was 26.1%DM, in both treatments. The experimental design was a randomized block design, using parity, milk yield, and DIM data from pre-experimental period as blocking factors. Statistical analyses were performed using the GLM for single measures and MIXED procedure of SAS for repeated measures over time. The results indicate that cows fed finely ground corn diet had lower ($P < 0.01$) fecal starch than cows fed coarsely ground corn diet (4.84 vs. 6.96%; SEM = 0.26), as well as greater ($P < 0.01$) total-tract starch digestibility (93.95 vs. 91.29%; SEM = 0.32). Individual dry matter intake was also lower ($P < 0.01$) for cows fed finely ground corn diet (21.22 vs. 22.05 kg; SEM = 0.20). Milk yield tended to be greater for cows fed finely ground corn diet (37.90 vs. 36.85 kg; SEM = 0.45; $P = 0.12$), as well as greater actual milk feed efficiency (1.78 vs. 1.69 L/kg; SEM = 0.04; $P = 0.15$), than for cows fed coarsely ground corn diet. However, milk fat (3.63 vs. 3.73%; SEM = 0.07; $P = 0.29$) and milk total protein (3.31 vs. 3.34%; SEM = 0.03; $P = 0.52$) contents, respectively for finely and coarsely ground corn diets, were not affected by treatment, as well as lactose, casein and total solids contents. Plasma D-lactate concentration was greater ($P = 0.04$) for cows fed finely ground corn diet (98.5 vs. 79.7 µM; SEM = 6.10). In conclusion, the adoption of smaller dry ground corn mean particle size improves starch availability and decreases fecal starch, improving total-tract starch digestibility. Furthermore, reducing dry ground corn particle size decreased DMI and improved milk production efficiency.

Key Words: dry matter intake, milk yield, starch digestibilty

T128 Performance of early lactation dairy cows receiving doses of calcium salts of palm oil supplemented or not with lysolecithin.

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Our objective was to evaluate the effects of supplementation with doses of Ca salts of palm oil containing 0 (CSPO) or 1% lysolecithin (CSPOL) on production responses of early lactation dairy cows. Forty-four Holstein × Jersey crossbred cows, 16 primiparous and 28 multiparous, were used in a randomized complete block design. Cows were fed corn silage ad libitum plus 9 kg of concentrate (as fed). The Ca salts (Kemin Industries, Inc.) were incorporated into the concentrates. The experiment lasted 13 wk, from 4 to 16 wk postpartum. Treatments were concentrate with 400 g of CSPO (T1), or concentrates containing 3 levels of CSPOL: 280 g (T2), 340 g (T3), or 400 g (T4). Intake of DM, yields of milk and milk components, BW and BCS were measured for individual cows. Data were

Table 1 (Abstr. T128). Performance of cows supplemented or not with lysolecithin

	Treatment					Contrast		
Item, kg/d	T1	T2	T3	T4	SEM	Lyso	L	Q
Intake								
Silage	10.3	10.8	10.4	10.2	0.2	0.77	0.07	0.13
DM	18.2	18.6	18.2	18.0	0.2	0.77	0.07	0.13
Milk	24.0	24.6	24.9	25.3	0.9	0.19	0.48	0.86
ECM	25.0	25.6	25.8	25.4	1.1	0.58	0.84	0.85
Fat	0.91	0.95	0.88	0.93	0.03	0.82	0.63	0.13
Protein	0.74	0.75	0.76	0.75	0.03	0.59	0.93	0.90
BW change	1.77	0.65	1.20	2.00	0.8	0.61	0.27	0.41

analyzed by ANOVA with mixed models using the MIXED procedure of SAS. Preplanned contrasts were: effect of lysolecithin (Lyso, T1 vs. T4), and the linear (L, T2 vs. T4) and quadratic (Q, T3 vs. 1/2 T2 + 1/2 T4) effects of increasing the amount of CSPOL. No interaction between treatment and parity was observed for any of the responses analyzed. Addition of lysolecithin did not affect intake, but increasing the dose of CSPOL tended to reduce forage intake, which affected daily DMI (Table 1). Treatment did not affect yields of milk and milk components or change in BW. We conclude that the Ca salts of palm oil, containing 1% lysolecithin, did not affect milk production and the energy partition in dairy cows.

Key Words: emulsifier, lysolecithin, fat supplementation

T129 Crosslinked gelatin capsules containing fish oil compared to untreated capsules improved milk fat yield when fed or directly placed into the rumen of Holstein cows. O. M. Peña*[1], S. Saunier[1], K. Murphy[2], G. L. Lascano[1], M. J. Aguerre[1], and T. C. Jenkins[1], [1]*Department of Animal and Veterinary Sciences, Clemson University*, *Clemson, SC*, [2]*Virtus Nutrition LLC*, *Corcoran, CA*.

The objective of this study was to determine the effect of fish oil in treated versus untreated gelatin capsules on milk fat yield and to establish if there was any difference between feeding capsules versus their direct placement into the rumen. Four multiparous ruminally-fistulated Holstein cows were randomly assigned to 1 of 4 treatments in a 4 × 4 Latin square design with 21 d periods. Treatments were: 1) no capsules (CON), 2) untreated capsules (UG), 3) treated capsules fed with the TMR (TGF), and 4) treated capsules placed directly into the rumen (TGR). Both, TGF and TGR capsules were treated with alcoholic solutions of flavoring agents followed by drying. Cows were fed twice a day a TMR with 52% forage and 48% concentrate (DM basis), and formulated to contain 16.7% CP, 29.3% NDF, and 26.2% starch and were milked twice daily. Data were analyzed using JMP Pro 12 with the following preplanned orthogonal contrasts: (1) CON vs. (UG + TGF + TGR), (2) UG vs. (TGF + TGR) and (3) TGF vs. TG. Milk yield (49.6 ± 2.49 SEM kg/d) and DMI (28.4 ± 0.49 SEM kg/d) were not affected by experimental treatments. Fat percentage was higher ($P < 0.02$) for CON compared with UG + TGF + TGR (3.40 vs. 2.86%) and higher ($P < 0.07$) for treated vs UG capsules (3.00 vs. 2.60%). Milk fat yield was also higher ($P < 0.01$) for CON vs fish oil capsules (1.71 vs 1.44 kg/d, $P < 0.01$) but there was not difference between delivery method for treated capsules. Ruminal concentrations of 20:5n-3 and 22:6n-3 tended ($P < 0.20$) to be lower for TGF and TGR compared with UG, indicating the treatment process slows ruminal release of fish oil. When capsules were fed, trans-10 fatty acid concentration in milk tended ($P < 0.07$) to be higher compared with CON (2.52 vs. 1.29%). Feeding fish oil capsules negatively affected milk fat synthesis. However, results from this study suggest that treated capsules provided limited ruminal protection and partially prevented digestion, decreasing the effect on ruminal biohydrogenation.

Key Words: milk fat, omega-3 fatty acid, biohydrogenation

T130 Effects of choline and docosahexaenoic acid on the lipidome of bovine precision-cut liver slices cultured with palmitic acid. J. E. Rico*[1], B. N. Tate[1], W. A. Myers[1], V. Sáinz de la Maza-Escolà[1,2], C. Chang[1], L. F. Wang[1,3], and J. W. McFadden[1], [1]*Cornell University*, *Ithaca, NY*, [2]*University of Bologna*, *Bologna, Italy*, [3]*Henan Agricultural University*, *Zhengzhou, China*.

Fatty acids (FA) and choline are involved in phospholipid synthesis and may influence hepatic triglyceride (TG) deposition and secretion in transition cows. Liver was biopsied from pregnant Holstein dry cows (parity = 2.4 ± 0.5) and precision-cut liver slices (~200 µm) were incubated in serum-free M199 medium on titanium inserts under 95% oxygen/5% carbon dioxide within a roller culture incubator. Slices were either unsupplemented (no FA or choline), treated with 200 µM palmitic acid (PA), or treated with PA with 2 mM choline chloride (CC), 200 µM docosahexaenoic acid (DHA), or CC plus DHA for 8 h. Slice viability was assessed by measuring adenosine triphosphate (ATP) and lactate dehydrogenase leakage at h 0 and 8. Lipidomics was performed using mass spectrometry. For each experiment, 3 cows with 3 slices per cow were utilized (9 total reps/experiment). Protein-normalized, generalized log-transformed and auto-scaled data were analyzed using ANOVA. Partial least squares discriminant analysis (PLS-DA) was performed. Slice ATP and lactate dehydrogenase leakage was not different between h 0 and 8; therefore, slices were deemed viable. Lipidomics revealed 256 lipids including but not limited to TG and phosphatidylcholines (PC). The PLS-DA model distinguished slices treated with CC, DHA, or CC with DHA in the presence of PA. For component 1, changes in TG and PC were observed in response to treatment (variable importance of projection scores >1.95). Enrichment of 16:0 and 22:6 within TG was observed in slices treated with PA or DHA (e.g., TG 15:0/16:0/16:0 and TG 16:0/22:6/22:6, respectively; false discover rate [FDR] < 0.001). Treatment with PA increased PC 31:0 and 32:0; however, CC abolished these effects (FDR <0.001). Treating slices with CC or DHA increased many unsaturated PC (e.g., PC 31:1, 36:3, and 37:2; FDR <0.001), relative to PA. However, in the presence of PA, unsaturated PC were often lower with CC and DHA co-treatment, relative to DHA alone (e.g., PC 36:6; FDR <0.001). We conclude ex vivo that the ability of CC to modulate liver slice PC status depends on DHA availability.

Key Words: cow, lipidome, liver slice

T131 An upgrade of the neutral detergent fiber characterization in NDS Professional. E. Raffrenato*, A. Ferrari, and E. Melli, *RUM&N Consulting*, *Reggio Emilia, Italy*.

Neutral detergent fiber degradation has recently been described by a 3-pool system, with the contemporary exponential decay of a fast (B3) and slow (B4) pool, their rates (k_{B3}, k_{B4}) and an indigestible pool (C). These fractions are now correlated with intake in ration evaluations and they will be implemented in CNCPS. Their accurate and precise characterization is therefore of utmost importance. The aim of this study was to validate and, possibly, improve NDF characterization done by a Vensim model (V) as described in Raffrenato et al. (2019), using 3 time points NDFd. A set of 500 fermentation profiles was randomly generated, each corresponding to a unique combination of B3, B4, C, k_{B3} and k_{B4}, based on published ranges. An alternative algorithm was developed using the same 3 time points (M1). The true values were regressed on the ones predicted by V and M1 and RMSPE was calculated. The significance of the deviation of the intercept from 0 and the slope from 1 was analyzed by *t*-test. The V model resulted in weak 3-time point predictions, with the risk of picking a local, vs. a global, minimum payoff. We then allowed the addition of the 12-h NDFd to the model (M2). Both R^2 and RMSPE indicated that both M1 and M2 performed better than the V model, with M2 performing better than M1. The payoff was numerically smaller for M1 and M2 in 482 samples, and very similar to the V payoff in the remaining cases. The V model had R^2 always lower than 0.60 with M1 and M2 having R^2 higher than 0.80 and 0.90, respectively. The V model had also slope larger than 1 ($P < 0.05$) for k_{B3}. The V model consistently underestimated the aggregated kd, resulting in positive residuals, especially for the lower quality samples. The risk of having a false low value is then higher than when predicting a higher kd forage. M1 residuals were independent of the size of the kd and M2 residuals confirmed the drastic improvement of the prediction when including the 12 h time point NDFd in the prediction. The study clearly showed how relevant is NDFd before 30 h, especially for extreme cases of either very high or very low-quality forages. Both M1 and M2 are now implemented in NDS Professional (RUM&N, Italy).

Key Words: modeling, CNCPS, rate of degradation

T132 Effects of abomasal infusions of fatty acids and one-carbon donors on apparent fatty acid digestibility and absorption in lactating cows.

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Our objective was to determine the effects of abomasal infusion of 16- and 22-carbon fatty acids (FA) on apparent FA digestibility. Five rumen-cannulated Holstein cows (214 ± 4.9 DIM; 3.2 ± 1.1 parity) were enrolled in a 5 × 5 Latin square experiment with each experimental period lasting 6 d and 8-d washouts. Abomasal infusates consisted of (1) palmitic acid (PA; 98% 16:0 of total fat), (2) PA + choline chloride (PA+C; 50 g/d choline chloride), (3) PA + l-serine (PA+S; 170 g/d l-serine), (4) behenic acid (BA; 92% 22:0 of total fat), and (5) docosahexaenoic acid algal oil (DHA, 47.5% DHA of total fat). Emulsions were formulated to provide 301 g/d of total FA and were balanced to provide a minimum of 40 and 19 g/d of 16:0 and glycerol, respectively. Cows were fed a corn silage-based diet at 115% of expected intake. Diet was sampled (d 4–6) and composited. Feces were collected every 8 h on d 6–7 and composited for analysis. Diet and feces were stored in sealed plastic bags at −20°C until dried. Indigestible NDF was used as an internal marker to estimate fecal output and apparent nutrient digestibility, and was estimated as NDF residue after 240 h in vitro fermentation. Fatty acids were analyzed by GC-FID and grouped by carbon chain length. Data were analyzed under a mixed model including the effects of period, cow and treatment. Fisher's LSD mean separation was performed when *P*-value in F-test <0.05. Intake of 22C FA was higher in BA and DHA relative to PA, PA+C, and PA+S, whereas 16C intake was higher in PA, PA+C, and PA+S, relative to BA and DHA ($P < 0.05$). Digestibility of DM was highest in PA and DHA and lowest in BA ($P < 0.05$). Digestibility of FA was highest in DHA, intermediate in PA, PA+C, and PA+S, and lowest in BA ($P < 0.05$). Digestibility of 16C was lowest in BA and highest in PA, whereas 18C digestibility was lowest in BA and highest in DHA ($P < 0.05$). As anticipated, 22C digestibility was highest in DHA, relative to BA (99% vs. 58%, respectively, $P < 0.01$). We conclude that FA chain length and degree of saturation affect FA availability for absorption.

Key Words: cow, digestibility, fatty acid

T133 Evaluating the effects of feeding whole cottonseed on primiparous and multiparous lactating dairy cows.

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Milk fat is a valuable milk component and around 60% of milk fatty acids (FA) originate from dietary fat. Oilseeds are an economical source of oil compared with dry fat supplements, but contain unsaturated FA that increase risk for diet-induced milk fat depression. Cottonseed is expected to have a slower ruminal release rate of unsaturated FA and decrease diet-induced MFD. The hypothesis of the experiment was that increasing whole cottonseed in the diet would increase milk fat yield in high producing cows by providing additional dietary fat without induction of milk fat depression. Four primiparous and 8 multiparous lactating Holsteins were arranged in a 4 × 4 Latin square design with 21 d periods. Treatments were 0, 3.3, 6.6, and 9.9% whole cottonseed. Dietary NDF and protein were maintained by substitution for cottonseed hulls and soybean meal. Data were analyzed using the random effect of cow and period and fixed effect of treatment and preplanned contrasts tested the linear and quadratic effect of increasing cottonseed level. Milk yield was not modified by treatment. There was a treatment by parity interaction for milk fat percent and yield with no effect of cottonseed in multiparous cows, but milk fat percent and yield were quadractically decreased in primiparous cows. Importantly, multiparous cows were 4.05% and primparous cows were 3.53% milk fat when fed the control diet. Milk FA profile indicated that cottonseed increased trans-10 C18:1 in primparous, but not multiparous cows. Milk fat increased at the highest level of cottonseed in primiparous cows due to an increase in yield of preformed FA. In conclusion, cottonseed maintained milk and milk component yield when fed at up to 9.9% of the diet to multiparous cows, but the primparous cows in the current trial were more susceptible to diet-induced milk fat depression.

Key Words: milk fat, cottonseed, milk fat depression

T134 The effect of a unique high-fatty-acid supplement on milk fatty acid profile and energy utilization of lactating Jersey cows.

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Omega-3 fatty acids are polyunsaturated fatty acids that play an important role in human health. To improve dietary consumption of omega-3 fatty acids, enrichment of dairy products through selective feeding of feedstuffs high in omega-3 fatty acids to dairy cows may be an option. Four multiparous Jersey cows (115 ± 36 d in milk) were used in a 4 × 4 Latin square with 4 periods of 28 d (24 d adaptation and 4 d collection) to analyze the effect of feeding a high fatty acid supplement on the milk fatty acid profile and energy utilization. Cows were randomly assigned to 4 different diets ranging from 0 to 20.0% inclusion of Perfect Omega 3 (PO3) (Sunseo Omega 3; Chungcheong Buk-Do, South Korea), a feed supplement comprised of sesame meal, giant kelp, cassava, and sorghum. With increasing inclusion of PO3, dry matter intake tended to increased linearly ($P = 0.108$) from 21.8 to 24.1 ± 1.41 kg/d, while milk yield did not differ ($P > 0.310$, averaging 31.4 ± 0.37 kg/d). Milk fat percentage increased linearly from 5.30 to 5.82 ± 0.35% with increasing inclusion ($P = 0.024$), and milk fat yield had a tendency to increase linearly ($P = 0.154$) from 1.66 to 1.85 ± 0.20 kg/d. Increasing inclusion of PO3 resulted in a linear increase ($P < 0.001$) in the concentration of α-linolenic acid in milk fat from 0.24 to 0.72 ± 0.04 g/100 g of milk fat as well as a linear decrease ($P = 0.002$) in linoleic acid from 2.61 to 2.23 ± 0.09 g/100 g milk fat. The increasing inclusion of PO3 linearly increased ($P = 0.067$) the GE content of the diet, but there was no difference ($P > 0.192$) among diets in DE and ME (averaging 60.9 ± 2.67 and 53.9 ±

2.57 Mcal/kg of DM). No difference ($P > 0.383$) was observed in the digestibility of NDF and energy averaging 38.2 ± 1.85 and 62.7 ± 0.65%, respectfully. The results of this study indicate that increasing inclusion of PO3 maintains milk production while increasing the concentration of milk fat, with favor toward a greater concentration of omega 3 fatty acids.

Key Words: polyunsaturated fatty acids, energy utilization, indirect calorimetry

T135 Low starch diets improve dry matter intake and energy balance in early lactating Holstein dairy cows. N. Juliano*[1,5], M. F. Olmeda[2,5], L. M. Buraschi[3], M. V. Dunleavy[4], F. Bargo[5], and R. A. Palladino[1,5], [1]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)*, *Buenos Aires, Argentina*, [2]*Universidad de Lomas de Zamora*, *Buenos Aires, Argentina*, [3]*Universidad Nacional de Mar del Plata*, *Buenos Aires, Argentina*, [4]*Instituto de Patobiología, Instituto Nacional de Tecnología Agropecuaria*, *Buenos Aires, Argentina*, [5]*PROLACT (UNLZ-FIL)*, *Buenos Aires, Argentina*.

The aim of this study was to evaluate low starch (LS) and high starch (HS) diets and its effect on DMI, energy balance (EB), milk yield and composition, energy-corrected milk (ECM), BW and body condition score (BCS, 1–5 scale) changes in early lactating Holstein dairy cows. Twenty-four Holstein cows (12 multiparous, 554.61 ± 25.12 kg BW; 12 primiparous; 568.63 ± 23.52 kg BW; mean ± SD) were assigned to 1 of 2 treatments at calving during 28 d: LS and HS. Diets consisted in corn silage plus pelleted concentrate formulated to be iso-energetic and iso-nitrogenous, on a 50:50 forage to concentrate ratio. Dietary treatments were adjusted by altering concentrations of corn grain and soyhulls to reach a target of 22 and 28% starch for LS and HS respectively. The DMI and milk yield were measured daily and milk composition, BW and BCS were measured weekly. Blood samples were taken from the jugular vein at 1, 7, 14, 21 and 28d since calving and analyzed for glucose, NEFA, BHB, insulin and IGF-1. The EB was calculated using DMI, energy consumption, BW changes and the estimated requirements based on milk production and composition (NRC, 2001). Data were analyzed using the mixed procedure of SAS (University Edition, 2018), as a randomized complete block design, with the animal nested to treatment as random effect, parity, day, treatment and their interaction as fixed effects. Cows fed LS diet had higher DMI compared with HS (20.57 vs. 17.83 kg/d, $P < 0.01$), with no treatment x parity interaction. There were no differences ($P > 0.05$) in milk yield (25.03 ± 1.21 kg/d), ECM (27.26 ± 1.68 kg/d), fat % (3.97 ± 0.21%) and protein % (3.59 ± 0.07%), BW (536.49 ± 17.1 kg) and BCS changes (0.25 ± 0.12 units) between treatments. No differences between treatments were found for blood metabolites and hormones. Cows fed LS diet had a less estimated negative EB with shorter duration (-0.06 vs. -4.22 Mcal NEl/d; 14 vs 28d, $P < 0.05$). These results show that lowering starch levels in early lactation diets has a positive effect on both DMI and EB, and may shorten the negative EB period.

Key Words: energy balance, starch level, transition period

T136 In situ effective ruminal dry matter and neutral detergent fiber disappearance of canola meal from twelve Canadian crushing plants over four production years. E. M. Paula*[1], J. L. P. Daniel[2], L. G. Silva[3], G. M. Wachekowski[1], H. H. A. Costa[4], and A. P. Faciola[5], [1]*Instituto de Zootecnia, Centro APTA Bovinos de Corte*, *Sertaozinho, SP, Brazil*, [2]*Universidade Estadual de Maringa*, *Maringá, PR, Brazil*, [3]*Department of Animal Science, College of Agrarian and Vet Sciences*, *Jaboticabal, SP, Brazil*, [4]*Universidade Estadual Vale do Acarau*, *Sobral, CE, Brazil*, [5]*University of Florida, Animal Sciences Department*, *Gainesville, FL*.

Canola meal (CM) has been widely used as protein source for dairy cows; however, CM contains about 30% NDF (% of DM), which is greater than other vegetable plants used as protein sources, such as soybean meal. According to current prediction models CM NDF ruminal digestibility can be as low as 35%, which may not accurately estimate CM energy value. Therefore, the objective of this study was to assess in situ effective ruminal DM and NDF disappearance of CM produced in Canada over a 4-year period. Samples were collected from 12 Canadian processing plants over 4-years (2011–2014, n = 48). Ruminal in situ incubations were performed at 0, 24, 48, 96 and 288h to determine NDF fractions A, B, and C and the effective ruminal DM and NDF disappearance (ERD). Approximately 1.25 g of sample was weighted, in triplicate, into Dracon bags (R510, 5 × 10 cm, 50 µm porosity, ANKOM Technology), consisting of an area ratio: surface of 20 mg/cm^2/sample. Bags were incubated in reversal chronological order in the ventral rumen of 3 cannulated steers. Statistical analyses were performed using PROC Glimmix of SAS. The model included processing plant and year of harvesting as fixed effects. Processing plant did not affect dry matter ERD ($P = 0.37$) and averaged 87.5% of DM. However, CM harvested in 2011 and 2013 had greater values than 2012 and 2014 (90.1 vs. 84.95 of DM, on average). Processing plant affected ($P < 0.01$) NDF fraction A and B, where for fraction A the lowest value was 12.2 and the highest 33.4% of NDF, and for fraction B the lowest value was 45.8 and the highest 70.2% of NDF. Fraction C and neutral-detergent fiber ERD (NDFERD) was not affected by processing plant and averaged 19.9 and 76.8% of NDF, respectively. Similarly, with dry matter ERD, NDFERD was affected ($P < 0.01$) by year of harvesting, where 2011 and 2013 had greater values than 2012 and 2014 (80.4 vs. 73.2% of NDF, on average). Overall, our results indicate that NDF ruminal digestibility of CM can be as high as 82.4% and, therefore, the current prediction models may underestimate CM energy value.

Key Words: energy, fiber digestibility, indigestible NDF

T137 Effects of *Ascophyllum nodosum* meal and monensin on ruminal fermentation and microbiota. L. H. P. Silva*[1], A. T. O. Melo[1], S. F. Reis[2], B. P. Jackson[3], F. Evans[4], and A. F. Brito[1], [1]*University of New Hampshire*, *Durham, NH*, [2]*Faculdade de Ciências Biomédicas de Cacoal*, *Cacoal, RO, Brazil*, [3]*Dartmouth College*, *Hanover, NH*, [4]*Acadian Seaplants Ltd*, *Dartmouth, NS, Canada*.

Ascophyllum nodosum meal (ASCO) is known to contain high concentrations of phlorotannins and iodine, which can impact the ruminal microbiota. However, there are few studies that have evaluated how ASCO affect ruminal microbiota. Five ruminally-cannulated Jersey cows were used in a 5 × 5 Latin square design to evaluate the effects of incremental levels of ASCO (0, 57, 113, and 170 g/d) or monensin (MON; 300 mg/d) on ruminal fermentation and microbiota. Each experimental period lasted 28 d with 21 d for diet adaptation and 7 d for sample collection. Cows were housed in a freestall equipped with Calan doors. A basal diet with a 65:35 forage:concentrate ratio (DM basis) was offered twice daily. Ruminal fluid was collected 3 h after the morning feeding for 3 consecutive days (d 23–25) of each sampling period and pooled for protozoa count, total bacteria, and microbiota analysis. Ruminal fluid was also collected at 0, 1, 2, 4, 6, and 8 h after feeding on the d 27 for ruminal pH, NH_3-N, and VFA analysis. Total bacteria were quantified by RT-qPCR. Microbiota analysis was performed on a MiSeq platform and processed using QIIME2. All data were checked for normality by Shapiro-Wilk test, and when necessary log-transformed. Data were analyzed in SAS following a 5 × 5 Latin square design with repeated measurements for ruminal pH, NH_3-N, and VFA. Contrasts were used to 1) compare linear and 2) quadratic effects of ASCO levels, 3) ASCO vs. MON, and 4) control vs. MON. Ruminal pH and NH_3-N were not affected by treatments. Total VFA concentration in the rumen was not affected by MON but decreased linearly with ASCO supplementation ($P = 0.02$). Ruminal butyrate and the acetate:propionate ratio showed quadratic responses to ASCO ($P < 0.05$). Protozoa count and total bacteria were not changed by treatments, as well as bacterial and archaeal diversity. The protozoal Simpson diversity index and the protozoal genera *Entodinium* and *Isotricha* reduced linearly with incremental levels of ASCO ($P < 0.05$). Overall, ASCO did not affected protozoa count and total bacteria but reduced protozoal diversity with minimal effect on ruminal fermentation parameters.

Key Words: microbiota, rumen, seaweed

T138 Effects of OmniGen Pro on performance and rumen fermentation of Jersey heifers. Y. Jiang*, M. Garcia, H. A. Roberts, G. A. Blakeney, J. D. Chapman, and D. J. McLean, *Phibro Animal Health Corporation*, *Teaneck, NJ*.

OmniGen Pro (OGPRO; Phibro Animal Health, Teaneck, NJ) is a new OmniGen-family feed additive, developed to maintain the immunological foundation of OmniGen AF while improving rumen functionality and nutrient utilization. This study examined the effects of OGPRO on rumen fermentation of dairy heifers. Five ruminally cannulated Jersey heifers (275 kg BW) were included in a crossover design with 2 periods of 30 d and 17 washout days between the 2 periods. Heifers were randomly assigned to 1 of 2 dietary treatments Control (no additive) or OGPRO (top-dressed at 4.5 g/45.4 kg BW). A single TMR representing a late lactation diet (14.7% CP, 31% NDF, 21% starch, 1.56 Mcal/kg NE_L) was offered ad libitum throughout the experiment. Rumen fluid was collected weekly (7, 14, 21, 28 d, in each period), 4 h after morning feeding to quantify fermentation via VFA, ammonia-N, and pH analyses. Data were analyzed with the GLIMMIX procedure of SAS, with significance declared at $P \leq 0.05$ and trend at $0.05 < P \leq 0.10$. Supplementing OGPRO did not affect ($P > 0.40$) DMI, ADG, or feed efficiency. There were no treatment differences in ruminal pH, propionate molar proportions, branched-chain fatty acids concentrations and molar proportions, nor ammonia-N concentrations. However, feeding OGPRO increased ruminal concentrations of total VFA (134 vs. 117 m*M*, $P = 0.01$), acetate (89.5 vs. 77.6 m*M*, $P = 0.003$) and butyrate (19.1 vs. 15.3 m*M*, $P < 0.001$) and tended to increase the molar proportions of butyrate (14.3 vs. 13.0%, $P = 0.06$). The benefits of OGPRO on rumen fermentation parameters were evident as early as 7 d, and there were no effects of diet × day. These findings demonstrate that the inclusion of OGPRO in lactating diets improves rumen fermentation, suggesting greater availability of nutrients and potentially better nutrient utilization.

Key Words: OmniGen Pro, lactating diet, rumen fermentation

T139 Effects of OmniGen Pro on performance, digestibility, and rumen fermentation of Holstein heifers. Y. Jiang*, M. Garcia, H. A. Roberts, J. D. Chapman, and D. J. McLean, *Phibro Animal Health Corporation*, *Teaneck, NJ*.

OmniGen Pro (OGPRO; Phibro Animal Health, Teaneck, NJ) is a new OmniGen-family feed additive, developed to maintain the immunological foundation of OmniGen-AF while improving rumen functionality and nutrient utilization. This study aimed to examine the effects of OGPRO on performance, digestibility, and rumen fermentation of dairy heifers. Fourteen Holstein heifers (229 kg BW) were randomly assigned to 1 of 2 treatments: Control (no additive, n = 7) or OGPRO (top-dressed at 4.5 g/45.4 kg BW, n = 7). A single TMR representing a late lactation diet (14.7% CP, 31% NDF, 21% starch, 1.56 Mcal/kg NE_L) was offered ad libitum for 16 d before the start of the study and during the 56-d study. Dry matter intake was measured daily and BW recorded every other week. Rumen fluid (d 0, 14, and 28) was collected 4 h after morning feeding via rumenocentesis to quantify fermentation via VFA and pH analyses. On d 29 and 30, fecal grab samples were taken to estimate digestibility using $uNDF_{240}$ as an internal marker. Data were analyzed with the GLIMMIX procedure of SAS, with significance declared at $P \leq 0.05$ and trend at $0.05 < P \leq 0.10$. Treatment did not affect ADG, DMI or D14 rumen fermentation parameters ($P > 0.10$). At d 28, there were no treatment differences in ruminal pH, propionate and butyrate concentrations and molar proportions, nor ammonia-N concentrations. However, feeding OGPRO numerically increased ruminal concentrations of total VFA (133 vs. 115 m*M*, $P = 0.15$) and acetate (90.8 vs. 78.2 m*M*, $P = 0.14$) and significantly increased total branched-chain fatty acids (1.44 vs. 1.02 m*M*, $P = 0.04$), primarily via an increase in isobutyrate (0.80 vs. 0.51 m*M*, $P < 0.01$). In addition, OGPRO tended to increase total-tract DM digestibility (73.3 vs. 67.7%, $P = 0.08$) compared with Control. These findings suggest that the inclusion of OmniGen Pro in lactating diets may improve nutrient utilization by altering rumen fermentation and DM digestibility.

Key Words: OmniGen Pro, lactating diet, rumen fermentation

T140 Evaluation of in vitro fermentation methodology to characterize the aNDFom degradation of intensively managed pasture samples. M. Dineen*[1,2], B. McCarthy[2], and M. E. Van Amburgh[1], [1]*Department of Animal Science, Cornell University*, *Ithaca, NY*, [2]*Teagasc, Animal and Grassland Research and Innovation Centre*, *Moorepark, Fermoy, Co. Cork, Ireland*.

Numerous animal studies have shown that when forages of higher in vitro digestibility but similar NDF concentration are fed, significant increases in DMI and milk production can be achieved. In vitro NDF digestibility methods, utilizing multiple fermentation time points (30, 120 and 240 h) combined with composite decay models, have been developed to more accurately predict such outcomes. However, this methodology has not yet been fully applied to pasture-based systems. Thus, our objective was to evaluate the in vitro methodology described by Raffrenato et al. (2018, 2019) to characterize the aNDFom degradation of intensively managed pasture (IMP) samples. Forty-six samples categorized by spring, summer, autumn and drought conditions (n = 8, 22, 8, and 8, respectively) were fermented for 12, 30, 120, and 240 h, with residues subsequently analyzed for aNDFom using a 1.5-µm-pore-size glass microfiber filter. Data were analyzed in SAS, using PROC TTEST to determine differences between paired means, PROC CORR to derive Pearson correlation coefficients, and PROC GLM to analyze the effect of sample category. Results indicated that over 90% of the potentially digestible aNDFom was digested before 30 h of fermentation and that a 12-h time point combined with 30, 120, and 240 h, is likely to better describe the aNDFom degradation curve of IMP. In addition, mean undigested aNDFom at 240 h (% of NDF) was affected by IMP category ($P < 0.01$), whereby spring and summer were lowest (9.8% and 11.1%, respectively), autumn intermediate (15.5%), and drought highest (20.0%). The extent of in vitro aNDFom digestion was not reached before 240 h; however, a strong Pearson correlation coefficient between 120- and 240-h time points (0.98) indicated the potential to predict undigested aNDFom from the 120-h time point for this data set. Our results suggest that the in vitro methodology evaluated, combined with a 12-h time point, can be utilized to characterize the aNDFom degradation of IMP.

Key Words: pasture, neutral detergent fiber, digestibility

T141 Effect of betaine supplementation on rumen microbiome of mid-lactating Holstein dairy cows.

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The effect of betaine on rumen fermentation stems from its action as a source of available ruminal nitrogen or methyl groups which may affect bacterial communities. Thus, we hypothesized that betaine supplementation affects rumen microbiome. Three rumen-cannulated Holstein dairy cows were used in a 3 × 3 Latin square design with 3 8-d periods (1 d for bag placement and ruminal fluid collection, and 7 d for washout). Three treatments of betaine at 0, 100, and 200 g were placed into Dacron bags (each bag contain 0, 100 or 200 g) which were put into the rumen triplicate at each time point (0, 1, 1.5, 2, 3, 6, 12, and 24 h). All the bags were removed at time point 24 h for organic matter analysis. Ruminal fluid from time points 0, 3, 6, 12, and 24 h was collected for pH and microbiome analysis. The relative abundance of taxa were analyzed using a generalized linear mixed model. Ruminal pH, richness, Simpson's evenness, Shannon diversity index, and Simpson diversity index were analyzed using a linear mixed model. R was used for permutational multivariate analysis using a Bray-Curtis dissimilarity matrix. Signficance was declared at $P < 0.05$. Results revealed that betaine supplementation decreased ruminal pH, Simpson's evenness, and all diversity indices after time 0, but no treatment × time point interactions were observed. The mean relative abundance from overall 24 h sampling time points of *Prevotella* 1 and *Streptococcus* increased with 200 g betaine while the relative abundance of *Ruminococcaceae* decreased. Over the 24 h, the relative abundance of *Prevotella* 1 (17.4 vs 38.17% for 0 and 24 h, respectively) increased with 200 g betaine. Only with 200 g betaine, the time point effect was observed to increase the relative abundance of *Streptococcus* (treatment × time point interaction). Permutational multivariate analysis showed that the variance of animal and treatment affected β diversity. Overall, betaine supplementation decreased ruminal pH, caused the bacterial taxa to shift toward starch utilizing bacteria (e.g., *Prevotella*) rather than cellulolytic bacteria (*Ruminococcaceae*), and led to decreased microbial diversity.

Key Words: betaine, ruminal pH, ruminal microbiome

T142 Using near infrared spectroscopy versus in vitro procedures for predicting dry matter digestibility.

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Total-tract dry matter digestibility (TTDMD) of a dairy cow ration can be determined using undigested neutral detergent fiber at 240 h as an internal marker. The objective of this study was to compare TTDMD calculated from neutral detergent fiber digestibility at 240 h ($NDFD_{240}$) analyzed by near-infrared spectroscopy (NIR) and in vitro methods. Fecal grab samples (n = 13) were collected for 2 consecutive days on 8 prepartum Holstein cows. Total mixed ration (TMR) samples were collected the day before and the first day of fecal sample collection to reflect the manure that was sampled. Corresponding orts samples were collected for each cow. All TMR, orts, and fecal samples were analyzed for dry matter and $NDFD_{240}$ by NIR and in vitro procedures at a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA). Individual cow intakes were recorded and TTDMD were calculated using $NDFD_{240}$ values obtained by NIR and in vitro methods. The TTDMD were calculated using both methods and were compared in SAS v.9.4 using a paired T-Test (PROC TTEST) and Lin's concordance correlation coefficient (CCC) was calculated. The CORR procedure was used to assess the correlation between the means and the differences of the 2 methods. The CCC yielded poor agreement (CCC = 0.40; 0 = no agreement, 1 = perfect agreement) between the 2 methods. The paired T-Test revealed that there was not a mean bias between the 2 methods (mean difference = −0.04; $P = 0.92$); however, the correlation between the bias (the difference between the methods) and the magnitude (mean of the methods) was significant ($r = -0.43$; $P < 0.0001$) indicating that the difference between the 2 methods is not random as the magnitude changes. Although NIR is a cost-effective and faster method, these results indicate that in vitro wet chemistry methods should continue to be considered the gold standard method for measuring $NDFD_{240}$ when calculating TTDMD.

Key Words: dry matter digestibility, method comparison, neutral detergent fiber digestibility

T143 Characterization of the dairy cow rumen epimural microbiota under high-forage and high-starch diets.

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The ruminal microbial communities of dairy cattle are crucial for providing nutrition from otherwise host-inaccessible dietary components. The majority of rumen microbiological research has focused on communities associated with luminal solids and liquids. However, a third microbiota is found closely associated with the ruminal epithelium (the epimural microbiota). This epimural community serves an important role as the interface between the host and its symbiotic community. Despite its functional importance, there is little research exploring the epimural microbiota within the context of diet and energy harvest in dairy cows. Diet has been shown to alter luminal microbial communities, but how epimural communities respond to similar changes in diet is less well understood. To explore this, we used next-generation 16S rRNA sequencing to characterize the bacterial community associated with the epimural tissue of 13 lactating Holstein dairy cows fed high-starch (diet A) and high-forage (diet B) diets. A diet-crossover experimental design consisting of 2 groups, 2 diets, and 2 sampling periods, was used. Animals were randomly assigned to 2 diet-transition groups (A-B, n = 6; B-A, n = 7). Groups received a single diet for 8 weeks, followed by a 10-d transition period and a final 8-week diet-treatment. All epithelial samples were collected at the end of each 8-week period. Rumen epithelial tissue was collected from the ventral sac after partial rumen evacuation. Under both diets, bacterial communities were characterized by high abundances of operational taxonomic units (OTUs) from the phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Epsilonbacteraeota*, and *Spirochaetes*. Our results suggest that diet did not significantly impact epimural community richness (Chao's richness; $P > 0.10$) or diversity (Shannon's diversity;

$P > 0.10$). However, we found that diet-treatment led to bacterial communities significantly different in structure (Bray-Curtis; PERMANOVA $P < 0.05$) and composition (Jaccard; PERMANOVA $P < 0.05$). Of the OTUs which changed in abundance according to diet, an OTU classified to the family Succinivibrionaceae increased in abundance under a high starch diet ($P < 0.05$) while another OTU classified to the order Bacteroidales increased in abundance under a high fiber diet ($P < 0.05$). These diet-dependent differences in epimural microbial community likely have important consequences for host energy harvest and general health.

Key Words: rumen, epimural, microbiota

T144 Effect of oscillating dietary starch on metabolic and acidotic status of lactating dairy cows. L. E. Hernández-Castellano, L. L. Pereira dos Santos, and M. Larsen*, *Dept. Animal Science, Aarhus University-Foulum*, *Tjele, Denmark*.

Six lactating multiparous Holstein cows (150 ± 49 DIM) with rumen cannulas and intercostal arterial catheters were used in a replicated 3 × 3 Latin square with 3 levels of oscillating (OSC) dietary starch to study the effect on metabolic and acidotic status. From d 1 to 7, cows were fed ad libitum with a control diet containing 20% starch (DM basis); from d 8 to 14 diets oscillated from 20 to 28 (LO), 35 (ME), or 42% (HI) starch. At d 8 and 14, ventral and medial rumen fluid, blood, and urine samples were collected at –0.5, +1, +2.5, +4, +5.5, and +7 h relative to morning feeding. Data were analyzed with OSC level, day, time, and possible interactions as fixed effects. Time was considered repeated. Level of OSC did not affect DMI at either d 8 or 14 (20.4 ± 1.2 and 20.6 ± 1.3 kg; respectively; $P > 0.31$) or milk yield (30.5 ± 1.2 and 28.9 ± 1.2 kg, respectively; $P > 0.33$). Medial rumen pH was affected by OSC level ($P = 0.03$; OSC level × day × time interaction). In cows with HI oscillation, medial rumen pH decreased continuously at both d 8 and 14 from –0.5 h (5.66 ± 0.14 and 6.21 ± 0.14, respectively) to +7 h (5.13 ± 0.14 and 5.24 ± 0.14, respectively) relative to morning feeding. With LO and ME oscillation at d 8, medial rumen pH decreased from –0.5 h (5.69 ± 0.14 and 5.69 ± 0.14, respectively) and reached nadir at +4 h (5.54 ± 0.14 and 5.35 ± 0.14, respectively) relative to morning feeding. Similarly at d 14, cows with LO and ME oscillation medial rumen pH decreased from –0.5 h (5.89 ± 0.14 and 5.92 ± 0.14, respectively) and reached nadir at +2.5 h (5.40 ± 0.14 and 5.44 ± 0.14, respectively) relative to morning feeding. However, OSC level did not affect ventral rumen pH ($P > 0.17$), blood pH ($P > 0.15$) or urine pH ($P > 0.57$). Plasma glucose tended ($P = 0.07$) to increase proportionally with OSC level (3.87 ± 0.07, 3.96 ± 0.07 and 4.02 ± 0.07 with LO, ME, and HI oscillation, respectively), while OSC level did not affect plasma concentrations of BHB ($P > 0.38$) or D-lactate ($P > 0.11$). In conclusion, oscillating dietary starch decreased medial rumen pH, especially high level of starch oscillation, whereas blood and urine pH did not provide any indication of systemic acidosis.

Key Words: starch, oscillating, metabolism

T145 The effects of supplementing a seaweed, *Asparagopsis taxiformis*, to dairy cows on bacteria-archaea interactions in the rumen. D. Pitta[1], H. Stefenoni[2], N. Indugu*[1], M. Hennessy[1], B. Vecchiarelli[1], R. Shah[3], S. Garapati[4], C. Yarish[5], S. Welchez[2], S. Räisänen[2], D. Wasson[2], C. Lage[2], A. Melgar[2], M. Fetter[2], A. Hristov[2], [1]*University of Pennsylvania School of Veterinary Medicine*, *Kennett Square, PA*, [2]*The Pennsylvania State University*, *University Park, PA*, [3]*University of Pennsylvania*, *Philadelphia, PA*, [4]*Drexel University*, *Philadelphia, PA*, [5]*University of Connecticut*, *Stamford, CT*.

Research has shown that feeding marine algae, such as *Asparagopsis taxiformis* (AT), severely inhibits methanogenesis in the rumen; however, the impact of feeding AT on rumen microbes is unknown. To determine the effect of feeding AT in dairy cows, 20 Holstein cows were randomly assigned to 4 [control (CON), 0.25% AT (LAT), 0.5% AT (HAT), and oregano leaves (ORE)] treatments in a replicated 4 × 4 Latin square design with four 28-d experimental periods. Toward the end of each period, ruminal samples were collected using a stomach tube and filtered through 3 layers of cheesecloth to separate the solid and liquid fractions. The solid fraction was extracted for DNA, PCR-amplified for the 16S rDNA gene of bacteria and archaea, sequenced and analyzed for bacteria and archaea diversity. The enteric methane emission data showed a decrease in the mitigation effect of AT over time (a 52–57% reduction in methane yield in experimental periods 1 and 2 for HAT and no statistical effect in P3 and P4). At the community level, both bacterial and archaeal communities were different *(*$P < 0.05$) between treatments. *Methanosphaera* differed between HAT and control with a significant reduction for HAT in P1, a smaller decrease in P2, and no difference from CON in P3 and P4, whereas *Methanobrevibacter* populations were unaltered. Butyrate-producing bacteria such as *Butyrivibrio*, *Moryella*, and *Eubacterium* were higher in HAT treatments compared with CON and ORE throughout the experiment. These findings indicate that AT selectively inhibits specific clades of methanogens such as *Methanosphaera,* which may have a greater share in total methane formation in the rumen than previously thought. Inhibition of methanogens was accompanied by significant alterations in the rumen bacterial populations revealing that alternate pathways of fermentation may be triggered with methane inhibition. This is the first study demonstrating the effect of AT on microbial interactions within the rumen and their relationship to methane emissions.

Key Words: methylotrophic methanogens, *Asparagopsis taxiformis*, dairy cow

T146 Effects of rumen hypomotility on microbial community composition. A. Palmonari, D. Cavallini*, G. Canestrari, G. Buonaiuto, S. Speroni, L. Campidonico, F. Ghiaccio, A. Formigoni, and L. Mammi, *Department of Veterinary Sciences, University of Bologna*, *Ozzano Emilia, Italy*.

Sudden drop in rumination time is usually defined as rumen hypomotility (RH), and these events could be related to different causes (i.e., dietary changes, environmental stresses, digestive pathologies, oestrus, lameness). The objective of this study was to evaluate, in high producing cows, the changes in rumen microbiota due to RH. Eight lactating Holstein cows were fed an acidotic diet (75% concentrates) for 28 d, once moved from the group to tie-stall. Rumen content was sampled before the animals were moved (T-3), once in the tie-stall (T0), and after 14 and 28 d since the experimental diet was fed. Rumen fluid was collected using an esophageal probe, and the first 500 mL of liquid was discarded to avoid saliva contamination. DNA was extracted according to an existing protocol, quantified using a NanoDrop system. V3-V4 region of the 16S rRNA gene was amplified using 341F and 805R primers plus Illumina adapter, then sequencing was performed on Illumina MiSeq according to the manufacturer's instructions. Raw sequences were processed using a pipeline with PANDAseq, QIIME2 and DADA2. High-quality reads were clustered into high-resolution operational taxonomic units (OTUs). Using taxonomic assignment, OTU tables were collapsed from phylum to genus level. Alpha diversity was computed using OTUs, Shannon index and Faith's diversity metrics. Beta diversity was estimated by computing weighted and unweighted UniFrac distances into Principal Coordinates Analysis (PCA). Statistical analysis was performed using R. Significant differences in α or β diversity were assessed by Wilcoxon test. Overall, 4 animals underwent RH. Obtained results showed that animals with RH had a different microbial community composition compared with the others ($P < 0.01$), still maintaining a higher number of OTUs than animals reacting differently to the acidotic diet. Moreover, variability across samples at different time points was lower ($P < 0.01$) in RH animals compared with the others, showing higher consistency during the whole trial. In conclusion, rumen hypomotility impacted rumen microbial ecosystem, which showed several differences in respect to not affected animals.

Key Words: rumen hypomotility, rumen microbiota

T147 Effects of a cashew nut shell extract on energetic metabolism and inflammatory biomarkers in transition dairy cows. B. M. Goetz*[1], E. A. Horst[1], E. J. Mayorga[1], M. A. Abeyta[1], S. Rodriguez-Jimenez[1], S. Carta[1], C. Hikita[2], T. Watanabe[2], and L. H. Baumgard[1], [1]*Iowa State University*, *Ames, IA*, [2]*Idemitsu Kosan Co., Ltd*, *Tokyo, Japan*.

Objectives were to evaluate the effects of cashew nut shell extract (CNSE, anacardic acid 59% and cardol 18%) on energetic metabolism and inflammatory biomarkers in periparturient Holstein cows. The formulated CNSE final granule contained 50% CNSE. Fifty-one multiparous cows were stratified by previous 305 ME and parity and assigned to 1 of 2 treatments 21 d before expected calving and continued until 28 DIM: (1) CON (control diet; n = 17) or (2) CNSE-5.0 (control diet and 5.0 g/d CNSE granule; n = 34). Following parturition, 17 cows from the CNSE-5.0 treatment were reallocated into a third treatment group: CNSE-2.5 (control diet and 2.5 g/d CNSE granule; n = 17) resulting in 3 total treatments postpartum: 1) CON, 2) CNSE-2.5 and 3) CNSE-5.0. Treatments were mixed with ground corn and top-dressed on the base TMR. Blood samples were obtained on d −14, −7, 3, 7, 14, 21, and 28 relative to expected calving. Effects of treatment, time, and treatment × time were assessed using PROC MIXED. Circulating glucose and insulin prepartum were similar between treatments. Following parturition, glucose (5.5%; $P = 0.02$) and insulin (19%, $P = 0.07$) concentrations from CNSE-fed cows decreased relative to CON. NEFA did not differ between treatments prepartum, however there was a quadratic response to increasing CNSE levels on circulating NEFA postpartum, as they were elevated in CNSE-2.5 relative to CON and CNSE-5.0 cows (31 and 23%, respectively; $P = 0.01$). No effects of dietary CNSE were observed for BHB concentrations prepartum. After calving, circulating BHB tended to increase in CNSE cows (14%, $P = 0.05$). Prepartum and postpartum BUN concentrations were unaltered by CNSE supplementation. Circulating serum amyloid A and lipopolysaccharide-binding protein peaked immediately after calving and progressively decreased with time but was unaffected by treatment. Cortisol was unaltered by treatment. In summary, CNSE supplementation mildly altered energetics (but without clinical relevance) in the periparturient period and had little to no effect on inflammatory metrics.

Key Words: periparturient, anacardic acid, methane

T148 Effect of cashew nutshell extract on nutrient digestibility and rumen pH when simulating close-up or fresh-cow conditions under in vitro conditions. C. Compton*[1], C. Hikita[2], T. Watanabe[2], T. C. Jenkins[1], G. J. Lascano[1], and M. J. Aguerre[1], [1]*Clemson University*, *Clemson, SC*, [2]*Agri-Bio Business Department, Idemitsu Koscan Co*, *Tokyo, Japan*.

The objective of this study was to determine the effects of cashew nutshell extract (CNSE, 59% anacardic acid and 18% cardol) on dry matter, neutral detergent fiber, total nitrogen digestibility (IVTDMD, IVNDFD, and TNd, respectively), and rumen fluid pH under in vitro conditions. The study was conducted as a randomized complete block design with 4 replicates per treatment incubated for 24-h during 4 incubation runs. Each incubation was inoculated with rumen contents collected from 3 ruminally fistulated cows fed either a close-up (CU; 15.1% CP, 38.1% NDF, and 20.8% starch) or a fresh-cow diet (FC; 16.4% CP, 31.8% NDF, and 28.0% starch) with substrate representing the respective diet. Treatments consisted of 4 levels of granulated CNSE formulated to contain 50% CNSE and added to the cultures in stepwise increments equivalent to 0, 2.5, 5, and 10 g of CNSE. Data were analyzed with the mixed procedure of SAS and pre-planned orthogonal contrasts to test for linear and quadratic effects. Overall, dietary treatments incubated to mimic FC condition had a higher IVTDMD (75.7 vs. 67.9% DM), IVNDFD (23.6 vs. 15.8% NDF) and TN (41.1 vs. 53.0% DM) compared with CU. Rumen pH after 24 h of incubation was similar for both dietary conditions (6.79 vs. 6.85, respectively). When mimicking CU conditions, increasing the level of CNSE tended ($P = 0.10$) to increase IVTDMD (67.8 to 69.7% of DM), but incremental levels of CNSE tended to decrease IVTDMD from 76.6 to 74.9% of DM, when mimicking FC conditions. A similar, but significant ($P < 0.03$), pattern was observed for IVNDFD. Adding CNSE to the diet had no impact on TNd (% DM) but we observed a quadratic response on pH when mimicking both CU and FC conditions. The highest pH (6.85) was observed at 2.5 g/cow/d level for CU conditions and the highest pH (6.99) for FC was observed at 5 g/cow/d level. Under the conditions of this study, adding CNSE to the diet had positive effects on DM and NDF digestibility in CU diets and pH of FC, but negatively impacts digestibility in FC. Further research is warranted to identify the optimal dosage of CNSE.

Key Words: digestibility, dairy, fermentation

T149 Effect of 3-nitrooxypropanol on total and metabolically active bacteria and archaea interactions in the rumen of dairy cows. D. Pitta[1], A. Melgar[2], N. Indugu*[1], V. Shabtai[1], M. Hennessy[1], B. Vecchiarelli[1], M. Kindermann[3], N. Walker[3], and A. Hristov[2], [1]*University of Pennsylvania School of Veterinary Medicine*, *Kennett Square, PA*, [2]*The Pennsylvania State University*, *University Park, PA*, [3]*DSM Nutritional Products*, *Basel, Switzerland*.

Research indicates that 3-nitrooxypropanol (3-NOP), a methane inhibitor under investigation, reduces enteric methane emissions by an average of about 24% in dairy cows. However, the effect of 3-NOP on rumen microbes is unknown. The purpose of this study was to investigate the effect of 3-NOP on total and metabolically active ruminal bacteria and archaea in dairy cows over a 12-week period. Whole ruminal contents of 8 ruminally-cannulated early-lactation dairy cows were sampled at 2, 6, and 10 h after feeding during wk 4, 8, and 12 of a randomized complete block design experiment in which 3-NOP was fed at 60 mg/kg feed dry matter intake. The solid ruminal fraction was extracted for DNA and RNA, PCR-amplified for the 16S rDNA gene of archaea and bacteria, sequenced and analyzed for bacteria and archaea diversity. Concentration of dissolved hydrogen (H_2) increased at wk 4 and 8 and then decreased in 3-NOP supplemented cows compared with control cows. There was a difference ($P < 0.05$) between DNA and cDNA-based bacteria and archaea communities revealing that bacteria and methanogens differ in their metabolic capacities. In both cDNA-based and DNA-based analysis, weighted UniFrac analysis (commonly present populations) revealed differences ($P < 0.05$) by treatment, week, and treatment × week in bacteria and archaea communities. Among methanogens, *Methanosphaera* and *Methanobrevibacter* differed between control and 3-NOP supplemented cows. Among bacteria, at the genus level, the most abundant lineages such as *Prevotellaceae* and *Succinivibrionaceae* increased at wk 4 and 8 and then declined whereas genera such as *Lachnospiraceae* and *Ruminococcaceae* followed a reverse pattern which correlated with the patterns observed for dissolved H_2 concentrations. It can be concluded that whereas 3-NOP inhibited methanogens, its effect on certain bacteria was observed based on the hydrogen accumulated as a result of methane inhibition by 3-NOP and not by a direct effect on the bacteria themselves.

Key Words: enteric methane, rumen microbe, dairy cow

T150 Investigating the effects of different soybean products on rumen microbial populations in dairy cows using in vitro fermentation. M. Hennessy[1], J. Bender[1], M. Leibstein*[2], B. Vecchiarelli[1], N. Indugu[1], S. Garapati[3], J. Toth[2], L. Baker[1], and D. Pitta[1], [1]*University of Pennsylvania School of Veterinary Medicine*, *Kennett Square, PA*, [2]*Oceanside High School*, *Oceanside, NY*, [3]*Drexel University*, *Philadelphia, PA*.

While several soybean (SB) products are commonly included in dairy cow rations, their specific effects on rumen microbial populations are not well understood. This study examined 9 diets: a basal diet made up of corn silage, haylage, and corn; the basal diet plus either SB meal, SB hulls, SoyPlus, raw SB, or roasted SB; and the basal diet plus a combination of either SB meal and SoyPlus, SB meal and SB hulls, or SB hulls and SoyPlus. Three dairy cows from farm A were sampled for rumen contents and these samples were combined and added as an inoculum to fermen-

Table 1 (Abstr. T151).

	Treatment					*P*-value
Item	HH	HL	LH	LL	SEM	Trt
Wk 7, g/kg $BW^{0.75}$						
N intake	2.7	2.9	2.3	2.4	0.31	0.51
N output	1.0	1.5	0.9	0.8	0.21	0.12
N balance	1.6	1.6	1.3	1.5	0.45	0.96
Wk 9, g/kg $BW^{0.75}$						
N intake	1.2	1.3	1.4	1.3	0.16	0.81
N output	1.5^{a}	1.2^{b}	1.7^{a}	1.2^{b}	0.12	<0.01
N balance	-0.4^{b}	0.3^{a}	-0.3^{b}	0.3^{a}	0.18	0.04
Wk 11, g/kg $BW^{0.75}$						
N intake	2.9^{a}	2.0^{b}	3.2^{a}	2.2^{b}	0.14	<0.01
N output	1.7	1.4	1.9	1.4	0.17	0.08
N balance	1.2	0.7	1.3	0.8	0.24	0.15

tation vials containing feed and artificial buffer, purged with CO_2, and incubated at 37°C for 6h with slow agitation. The same procedure was carried out with 3 dairy cows from farm B. Samples were collected before (0h) and after (6h) incubation and separated into liquid and solid fractions. These samples were extracted for DNA, PCR-amplified for the V1-V2 region of the 16S rDNA bacteria gene, sequenced on an Illumina platform, and analyzed for bacterial diversity. At the community level, weighted Unifrac distance-based analysis revealed differences in communities between ruminal fractions and time of incubation ($P < 0.05$). Within each fraction, at 6h, differences in bacterial communities were influenced by source of rumen inoculum, diet, and interaction between inoculum and diet ($P < 0.05$). Comparison of individual bacterial taxa between fractions using ANCOM revealed that the solid fraction had lower amounts of *Succinivibrionaceae* and higher amounts of *Oribacterium* ($P < 0.05$) whereas the liquid fraction contained higher amounts of *Veillonellaceae* and lower amounts of *Clostridium* ($P < 0.05$) in all diets with soybean products compared with the control diet despite differences in the source of ruminal inoculum. In vitro-based microbial analysis revealed that soybean products may influence bacteria involved with soluble carbohydrate and protein breakdown with no effects on fiber-digesting bacteria. However, the causality behind these effects of soybean products on ruminal microbiota needs to be investigated using in vivo experiments.

Key Words: microbiome, proteolytic bacteria, ANCOM

T151 Nitrogen metabolism of Holstein calves on high or low pre- and post-weaning feeding plan. G. Antunez*[1], C. Cajarville[1], C. Fernandez[1], L. Artus[1], J. Dayuto[1], F. Correa[1], G. Oleggini[2], and J. Repetto[1], [1]*Departamento Producción Animal de Veterinaria (IPAV), Facultad de Veterinaria, UdelaR, Libertad, San José, Uruguay*, [2]*CONAPROLE*, *Montevideo, Uruguay*.

The aim was to study the effects of the pre- and post-weaning nutritional plans on N metabolism of calves. Twenty 4 female Holstein calves (41 ± 0.5 kg BW; 6 ± 2 d old), were blocked by birthdate, and randomly assigned to 4 nutritional levels (Trt): 1) high pre- and high post-weaning (HH), 2) high pre- and low post-weaning (HL), 3) low pre- and high post-weaning (LH), or 4) low pre- and low post-weaning (LL). Calves had free access to water and starter (21% CP, 15% NDF). Milk replacer (MR; 25% CP, 20% EE; 12% of DM) was provided twice daily at 10% (LH and LL) or 20% (HH and HL) of initial BW, stepping-down the offer since d 49 MR until weaning (d 56). After weaning calves were fed with different amounts (H or L) of a diet (50% starter and 50% hay; 18% CP; 40% NDF). Feed intake was recorded daily, and total feces were collected during wk 7, 9, and 11 to determine N excretion (NE), N balance (NB), N efficiency (Nef), and N absorbed (Nab). Spot samples of urine were taken for 3 d on wk 7, 9, and 11 to determine urine volume and N excreted by urine by creatinine concentration. The mixed model of SAS was used, considering calf as experimental unit, Trt as fixed effect and block as random. Means were compared by Tukey ($P < 0.05$). On wk 9, calves on HH and LH had more NE than calves on HL and LL and had negative NB (Table 1). No differences were observed on Nef (wk 7: 56 ± 10.2%, $P = 0.89$; 9: −6 ± 10.2%, $P = 0.89$; 11: 37 ± 8.1%, $P = 0.73$) or Nab (wk 7: 91 ± 1.0%, $P = 0.82$; 9: 74 ± 4.1%, $P = 0.13$; 11: 86 ± 1.4%, $P = 0.15$). Weaning seems to have affected N metabolism of calves (wk 9), but a high nutritional plane after weaning allowed to recover N intake and N balance 3 wk after weaning.

Key Words: nitrogen, digestibility, heifer

T152 Oscillating dietary starch had minor effects on performance in Holstein cows. M. Larsen*, P. Lund, L. E. Hernández-Castellano, and M. R. Weisbjerg, *Department of Animal Science, Aarhus University-Foulum*, *Tjele, Denmark*.

The effect of oscillating dietary starch on performance was investigated in 72 Holstein cows for 5 weeks in a randomized design. Cows were blocked according to parity and lactation stage. Cows were loose-housed with automatic milking (AMS) and automatic feed registration. Dietary starch was either stable (STA) or oscillating (OSC) in 2-d intervals with ± 20 g/kg DM at either 190 (LO) or 230 (HI) g starch/kg DM in a 2 × 2 factorial arrangement. Diets contained (g/kg DM) 257 corn silage, 257 grass-clover silage, 138 AMS concentrate, 80 canola cake, 40 soybean meal, 14 minerals+vitamins, as well as 119 rolled barley and 95 dried sugar beet pulp with LO starch or 182 rolled barley and 32 dried sugar beet pulp with HI starch. Oscillation was made by substituting 64 g/kg DM of dried sugar beet pulp with rolled barley at each starch level. Milk samples were collected the last 2 d of each week. Rumen fluid (oro-ruminal) and blood was collected from 36 cows. Data were analyzed with wk 0 as covariate and block, starch level, oscillation, week (repeated within cow), and possible interactions as fixed effects. The AMS concentrate intake did not differ among treatments (2.6 ± 0.1 kg/d; $P > 0.30$). Total DMI was greater with OSC-HI and STA-LO compared with the 2 other treatments (23.5 vs. 22.9 ± 0.3 kg/d; interaction: $P = 0.02$). The ECM yield was greater with OSC-HI and STA-LO compared with the 2 other treatments (37.2 vs. 36.1 ± 0.5 kg/d; interaction: $P = 0.03$). The effects on DMI and ECM were minor and the pattern difficult to relate to oscillation.

Milk protein content was higher for HI compared with LO (3.50 vs. 3.45 ± 0.02%; $P = 0.02$). Milk fat content was higher for OSC compared with STA (4.01 vs. 3.85 ± 0.05%; $P = 0.01$). Rumen propionate proportion was greater with HI compared with LO ($P = 0.02$), and butyrate proportion tended to be greater with OSC compared with STA ($P = 0.09$). Plasma glucose did not differ among treatments ($P > 0.51$), FFA was lower with HI compared with LO ($P = 0.03$), and BHB was numerically greater with OSC compared with STA ($P = 0.16$). The effects on milk components could be related to differences in rumen and plasma metabolites.

Key Words: starch, oscillating, production

T153 Effect of circadian rhythm and frequency of feeding on bacteria and archaea populations in the rumen of dairy cows. C. Pappalardo*, M. Hennessy, B. Vecchiarelli, N. Indugu, J. Bender, T. Gleysteen, J. Toth, and D. Pitta, *University of Pennsylvania School of Veterinary Medicine*, *Kennett Square, PA.*

The makeup of the microbial community in the rumen has been shown to fluctuate over the course of the day relative to feed intake. However, these changes have not been well characterized, and the effect on these variations caused by feeding once or twice a day has not been elucidated. For this study, 2 groups of 11 Holstein dairy cows (fed once [SID group] or twice daily [BID group]) were selected. Rumen samples were obtained via stomach tube at 3 time points (SID: TP1 = 1–2h post feeding, TP2 = 10–11h post feeding, TP3 = 16–17h post feeding; BID: TP1 = 1.5–2.5h post AM feeding; TP2 = 4–5h post PM feeding; TP3 = 10–11h post PM feeding). Samples were filtered to separate the solid fraction, extracted for genomic DNA, PCR-amplified for the bacterial and archaeal 16S rDNA gene, sequenced on the Illumina MiSeq platform, and analyzed using QIIME2. Bacterial richness as estimated by number of observed species was higher in TP2 and TP3 than in TP1 in the BID group whereas bacterial diversity estimated using Shannon index was higher in TP2 and TP3 than in TP1 in the SID group. At the community level, weighted Unifrac analysis showed differences ($P < 0.05$) between groups for bacteria and a trend for archaea ($P < 0.06$). Differences in time points in both groups for both bacteria and archaea were significant in unweighted Unifrac analysis. *Firmicutes* were the dominant phylum in both groups at all time points but were more prevalent in the BID group. At the individual taxa level, the most abundant bacterial genera were *Prevotella*, *Clostridiales*, *Butyrivibrio*, *Lachnospiraceae*, and *Ruminococcus* which differed significantly ($P < 0.05$) between groups. In the BID group these lineages either gradually increased or decreased from TP1 to TP3; in the SID group, *Prevotella* was lower in TP2 than in TP1 and TP3 and the other abundant genera showed the opposite trend. Among archaea, *Methanosphaera* gradually increased from TP1 to TP3 in the BID group while in the SID group it was higher in TP2 than TP1 and TP3. These results indicate that differences in feeding frequency influence diurnal variations in the rumen microbiome.

Key Words: diurnal variation, feeding frequency, rumen microbiota

T154 Yerba-mate as feed additive and its effects on feed intake and digestibility. R. R. Lobo*[1,2], Y. A. Peña-Bermúdez[1], D. A. Rojas-Moreno[1], C. M. da Silva[1], L. L. Panosso[1], V. Benetel Junior[1], L. R. Ghussn[1], V. C. Mufalo[1], A. P. Faciola[2], R. S. B. Pinheiro[3], A. Berndt[4], and I. C. S. Bueno[1], [1]*Universidade de São Paulo*, *Pirassununga, SP, Brazil*, [2]*University of Florida*, *Gainesville, FL*, [3]*Universidade Estadual Paulista*, *Ilha Solteira, SP, Brazil*, [4]*Embrapa Pecuária Sudeste*, *São Carlos, SP, Brazil.*

Our objective was to evaluate levels of inclusion of yerba-mate (*Ilex paraguariensis*) extract (YME) as feed additive and its effects on feed intake and digestibility in sheep. For this project, we used 24 intact male lambs (23.3 ± 2.2 kg) as models. Animals were assigned into 6 blocks of 4 animals each, and animals within blocks were randomly assigned to treatments. Animals were fed a basal diet containing 60:40 concentrate:forage. Treatments were divided by levels of inclusion of YME (0, 10, 20, and 40 g/kg of DM) fed as TMR. The experimental period lasted 53 d, and daily individual feed intake was measured. On d 30, digestibility measurements started, animals were equipped with total fecal collector bags and samples were collected for 8 d (3 for adaptation and 5 for measurements). The experiment was carried out as a randomized block design and data were analyzed using the MIXED procedure of SAS, orthogonal contrast were evaluated (linear, quadratic, and cubic). A negative linear effect ($P = 0.03$) was observed for DMI throughout the experiment, probably due to the high concentration of tannins in YME. Intake of YME had a positive linear effect in the digestibility coefficient of DM ($P = 0.04$) and NDF ($P < 0.01$). A quadratic effect in EE digestibility ($P = 0.02$) was observed, with a large reduction in treatments up to 20 g/kg of YME inclusion. No effects were observed for OM, CP, and ADF digestibility. In conclusion, YME at 40 g/kg of DM reduces DMI; however, inclusion of up to 20 g/kg of DM could increase fiber digestibility and reduce EE digestibility.

Table 1 (Abstr. T154). Apparent digestibility of nutrients (%) with increased levels of yerba-mate extract

	Inclusion of YME (g/kg)					Contrast[1]		
Item	0	10	20	40	SEM	Lin	Quad	Cub
DM	75.2	74.6	77.0	77.6	0.95	0.04	0.99	0.21
OM	77.4	76.9	78.7	78.4	0.97	0.31	0.75	0.29
CP	82.2	81.4	81.9	83.2	0.72	0.25	0.25	0.66
EE	74.0	68.7	65.3	70.7	2.28	0.41	0.02	0.79
NFE	79.0	78.0	81.9	80.9	1.05	0.08	0.46	0.05
ADF	41.1	42.5	45.4	45.8	2.58	0.18	0.61	0.74
NDF	61.4	63.9	67.8	67.7	1.20	<0.01	0.07	0.33

[1] Values from orthogonal contrast (linear, quadratic, and cubic) are significant when $P \leq 0.05$ and tendency when $0.05 < P \leq 0.10$.

Key Words: *Ilex paraguariensis*, natural additive, ruminant nutrition

T155 In vitro dry matter digestibility of *Typha domingensis* using adapted rumen microbes. S. LeShure Ratiff* and R. Kohn, *University of Maryland College Park*, *College Park, MD.*

There is a shortage of usable firewood for cooking in rural Nigeria. Bio-digesters serve as an eco-friendly, inexpensive cooking fuel by converting common waste into valuable biofuel/biogas, such as methane. *Typha domingensis*, also known as cattails, has become a nuisance aquatic weed that overpopulates and pollutes Nigerian waterways. Utilizing cattails as substrate for digesters would help rid Nigerian waterways of this weed and relieve the firewood shortage. The source of inocula used to "seed" bio-digesters is also being considered, whether to use rumen fluid from cattle gut harvested at slaughter or microorganisms from already established digesters. Rumen fluid often yields a faster digestion rate than biogas digesters, possibly because of selection caused by faster passage rates in the rumen. We conducted an experiment to evaluate cattail digestion in vitro. The hypotheses were (1) wild-type rumen microorganisms may bring about faster digestion rates over time when compared with substrate-adapted digester microorganisms, and (2) faster organic matter flow rate would increase digestion rate of digesters. We compared the digestion rate and methane production for rumen fluid versus inocula from 2 bench-scale digesters. These digesters were previously conditioned to pre-bloom cattail and fed at 2 organic feeding rates (FR = 0.04 g/d or 0.08 g/d). Each of the 3 sources of inoculate used with 5 differing maturities of *Typha domingensis*. Forage digestion rate and extent of digestion were greatest for rumen fluid, but the slower FR digester yielded similar digestion rates as the rumen fluid for pre-bloom cattails. The slower FR produced the most methane as a percentage of organic matter, and had higher digestion rate than the faster FR digester. The faster FR did not explain why rumen fluid yielded faster organic matter digestion rates.

Key Words: *Typha domingensis*, cattails, anaerobic digesters

Animal Health 3: Mastitis

W1 Hoof-impact and slide measurements for common Ontario dairy farm floorings. J. E. French*[1], J. J. Thomason[2], T. C. Wright[1], and V. R. Osborne[3], [1]*Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada*, [2]*Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada*, [3]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*.

In the context of preventing lameness and injury from slipping, our objective was to characterize hoof impact and slide on 6 flooring types (FT) commonly used in Ontario dairy farms: DC: diamond-grooved concrete, EC: sanded epoxy-covered concrete, GR: grooved rubber mat, HR: high-profile rubber mat, LR: low-profile rubber mat, and TG: turf grass (Kentucky bluegrass/fescue mix). Surface hardness (SH) was measured on each FT using a Clegg drop hammer. Five pre-trained lactating Holstein cows were each walked over all 6 surfaces sequentially in a randomized order. Walking speeds were determined from 200-fps videos in MATLAB. A 3-axis accelerometer that was epoxied to the lateral claw of each hindfoot captured continuous horizontal (aH) and vertical (aV) accelerations at 2500 Hz during each trial. All aH and aV waveforms were inspected on-screen to identify irregularities that indicated hoof slipping. Impact events (from contact to the hoof being still) were isolated for 3–6 stances/trial, and peak decelerations (aHmax, aVmax) were determined in MATLAB. Each aH impact event was double integrated backward, from still to moving, to estimate normal hoof slide. Ranking aHmax and slide values gave separate indicators of relative grip/slip among surfaces. The influences on aHmax and aVmax of FT, SH, cow, speed and claw (and all significant higher-order factors) were assessed by ANOVA in SAS 9.4, after verifying data normality. SH positively affected aVmax ($P < 0.05$), but not aHmax ($P = 0.75$), which are expected results. aHmax was affected by FT ($P < 0.01$), strongly implicating differences in surface friction (not measured here). The ranking of surfaces (from low to high grip) on aHmax was: LR, EC, HR, GR, DC, TG, and on hoof slide was EC, GR, HR, LR, TG, DC, with only LR moving substantially, indicating that the variables measure similar aspects of surface properties. Unexpected hoof slips were only seen for 3 stances, 2 on turf and 1 on HR. The hoof-surface interaction can be assessed in subtle detail, and such data may be of use in preventing injury by slipping.

Key Words: dairy cattle, hoof-surface interaction

W2 Detecting the bacterial variation of recycled manure solids for use as bedding in freestalls. H. Wu*, N. Zheng, and J. Wang, *Laboratory of Quality and Safety Risk Assessment for Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China*.

Recycled manure solids (RMS) was separated from cow feces as bedding. However, the influence of bedding microflora on milk bacteria remains unclear. To investigate the variation of bacteria in samples of RMS process and compare the contribution to microbe in mastitis /healthy bedding to milk microbe. Three consecutive days in 2019, the samples of rectum feces from healthy /mastitis dairy cows were collected. All feces were mixed and then subjected to fecal mixing pool, extrusion, stove, sun-cure according to the RMS process. Samples were collected for each step. Meanwhile, the Mastitis/healthy cowshed bedding and mastitis/tank milk were collected in the farm of Hebei Province. All samples were used to investigate the presence of pathogenic bacteria of mastitis by qPCR mastitis screening kit. And the bacterial diversity of those sample was determined by the variable region of v3-v4 in the flora determined by MiSeq. The Qiime2 software, SPSS, Galaxy and R software were used to analyze the Miseq data. All pathogenic bacteria of mastitis were not found in all samples except the milk samples of mastitis. The microflora similarity of faces and bedding was low in the mastitis/healthy sheds. Moreover, Lefse results showed that bacterial proportion of feces from healthy dairy cows was significant different with those from mastitis cows. F16 (1.8 ± 1.3%), *Erysipelototrichaceae* (1.4 ± 1.4%) and *Coriobacteriaceae* (0.2 ± 0.2%) had a higher proportion in the feces of healthy dairy cows, while only *Streptococcaceae* (0.5 ± 0.1%) had higher proportion of mastitis feces ($P < 0.05$). The enrichment of microorganisms in rectal feces decreased during the RMS processed steps. Compared with rectal feces, there were a higher proportion of *Xanthomadaceae* (9.2 ± 2.2%), *Sphingobacteriaceae* (2.6 ± 1.4%), *Saprospiraceae* (4.4 ± 2.4%), *Phyllobacteriaceae* (6.7 ± 2.4%), *Flammeovirgaceae* (1.0 ± 0.6%), *Flavobacteriaceae* (9.1 ± 4.9%) and *Alteromonadaceae* (3.7 ± 2.1%) in the bedding samples. The predominant bacteria in milk was not abundant in feces or bedding materials. In general, RMS for use as bedding may not increase the incidence of mastitis in dairy cows.

Key Words: recycled manure solids, microflora

W3 Identifying factors associated with lameness and its impact on productivity in automated milking herds. R. D. Matson*[1], M. T. M. King[1], T. F. Duffield[2], D. E. Santschi[3], K. Orsel[4], E. A. Pajor[4], G. B. Penner[5], T. Mutsvangwa[5], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada*, [3]*Lactanet, Sainte-Anne-de-Bellevue, QC, Canada*, [4]*Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada*, [5]*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada*.

Voluntary milking is critical for success in automated milking systems (AMS); impaired gait (lameness) may negatively affect the ability and desire for cows to milk voluntarily. The objective of this study was to assess the effect that lameness has on the productivity of AMS herds and identify factors associated with lameness. From April to September 2019, 76 robot herds were visited, and data on barn design and farm management practices were collected. Data from AMS units were collected, along with milk recording data for the 6 mo period before farm visits. Farms averaged 99 ± 73 lactating cows, 2.3 ± 1.4 robot units/farm, 43.9 ± 9.0 cows/robot, 36.7 ± 4.7 kg/d of milk, a milking frequency of 3.0 ± 0.4x/d, and a herd-average SCC of 198.3 ± 88.1 (x1,000) cells/mL. Thirty percent (minimum of 30 cows/farm) were scored for body condition (BCS 1 = underconditioned to 5 = over conditioned) and gait (1 = sound to 5 = lame; with clinically lame ≥ 3: 28.6 ± 11.7%; and severely lame ≥ 4: 3.0 ± 3.2%). Univariable models were used to screen independent variables (as fixed effects) in mixed-effect linear regression models and variables with $P < 0.25$ were offered to multivariable models. Clinical lameness was 10.2 percentage points (p.p.) less prevalent on farms with sand bedding ($P < 0.01$) and tended to be 2.8 p.p. lesser for each additional time stalls were raked/d ($P = 0.07$) and 5.7 p.p. lesser for farms that built new barns vs. retrofitting existing barns ($P = 0.07$). Herd average milk yield/cow decreased with greater prevalence of clinical (−0.1kg/d for 1 p.p. increase; $P = 0.01$) and severe lameness (−0.8kg/d with doubling of prevalence from 3 to 6%; $P < 0.01$). Milk yield/robot decreased with a greater prevalence of clinical lameness (−7.1kg/d for 1 p.p. increase; $P = 0.01$). Lesser milking frequency was associated with a greater proportion of over-conditioned cows ($P = 0.04$). SCC was associated with a greater proportion of clinically lame ($P < 0.01$) and under-conditioned cows ($P = 0.05$). Overall, this study demonstrates that productivity and milk quality in AMS herds are optimized by maintaining mobility and body condition of cows.

Key Words: automated milking system, lameness, herd management

W4 Screening stable flies and house flies as potential vectors of digital dermatitis in dairy cattle. R. Thibodeaux*[1], J. Brady[2], S. Swiger[3], and B. Jones[1,2], [1]*Tarleton State University, Stephenville, TX*, [2]*Texas A&M Agrilife Research, Stephen-*

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Digital dermatitis is a bacterial infection that causes lesions above the heal bulbs on cattle hooves. *Treponema* bacteria are present on the lesions of an infected hoof. Transmission is suspected through mechanical exposure in dirty bedding areas, but insects as potential vectors have not been explored. Flies can lead to other diseases like mastitis. Thus, the vast number of flies on a dairy may contribute to the spreading of digital dermatitis. Therefore, the objective of this research was to determine if flies captured from a dairy farm known to have digital dermatitis carry *Treponema* bacteria on their legs and heads. Autoclaved mesh sweep nets were used to collect the stable flies (*Stomoxys calcitrans*) and house flies (*Musca domestica*) for transfer to a plastic crate with a fabric opening until transport to the lab. The heads and legs of the fly were cut off with a sterile scalpel to use in the DNA extraction. Flies (n = 417) had DNA extracted using a Qiagen Blood and Tissue Kit. DNA from one tube was qPCR assayed in triplicate to determine if any DNA from *Treponema* bacteria were present. Three sampling events were used to increase the chance that *Treponema* DNA was collected if present. The concentration of DNA extracted from samples was measured by using a Qubit 2.0 Fluorometer kit (Life Technologies, Carlsbad, CA). A plasmid with *Treponema phagedenis* gene sequence was used as a qPCR positive control because this species was most prevalent in digital dermatitis infections. The negative control used was molecular biology grade water. The plasmid was serial diluted 1:10,000 before use as a qPCR positive control. *Treponema* bacteria were not detected on any of the flies we tested. The DNA of treponemes may not have been detected due to the small sample size compared with the overall population, or that other species of flies need to be analyzed to broaden the possibility of detecting the DNA. Further analysis should be conducted to confirm if other flies may carry *Treponema* bacteria, and to explore other potential vectors of *Treponema* bacteria.

Key Words: digital dermatitis, *Treponema*, qPCR

W5 Skin transcriptome affected by digital dermatitis in lactating dairy cows.

F. Rosa[1], N. A. Carpinelli*[1], J. T. R. Carvalho[1,2], K. Mjoun[3], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University*, *Brookings, SD*, [2]*Animal Science Department, Universidade Federal de Lavras*, *Brazil*, [3]*Alltech Inc*, *Brookings, SD*.

Bovine digital dermatitis (DD) is a polymicrobial disease that can lead to an inflammatory response of the hoof, keratinization loss, and lameness. Therefore, we performed skin tissue biopsies from the center of lesion or healthy skin of lactating dairy cows to assess the impact of DD on the skin transcriptome. Multiparous Holstein dairy cows were selected based on their condition as clinically healthy (HDD, n = 7), or cows diagnosed with DD (n = 7). Lameness was assessed based on locomotion score (scale 1 to 5), and it was 1 ± 0 and 2.3 ± 0.8 (mean ± SD) for HDD and DD, respectively. The skin biopsies were performed using a sterile biopsy punch. All samples were immediately flash-frozen in liquid nitrogen. The RNA integrity number for all samples was 7.9 ± 1.1. The RNA samples were sequenced (NGS; Illumina, NovaSeq S4) at the University of Minnesota Genomics Center. Processed reads were aligned to the bovine genome using HISAT2. Differential transcript analysis was performed using a quasi-likelihood test in R-package (EdgeR) and False Discovery Rate <0.05 correction was applied. Genes mediators of a proinflammatory response including *IL1A*, *IL1B*, *IL1RAP*, *IL18RAP*, and the transcription factor *NFKB1* were upregulated ($P < 0.002$) in DD cows in comparison to HDD. Greater ($P < 0.03$) expression of *CXCL6*, *CXCL8*, and *CXCL13*, related to innate immunity and chemo-attractants, was observed in DD in comparison to HDD. The type II cytokeratins *KRT3* and *KRT4* were upregulated ($P \leq 0.002$) in the skin of DD cows compared with HDD; however, *KRT18* was downregulated ($P = 0.003$) in the DD group. Tight junctions related-genes including the claudins *CLDN2*, *CLDN5*, and *CLDN10*, were downregulated ($P \leq 0.004$) in the skin of DD cows in comparison to HDD. Our preliminary data suggest DD can alter skin paracellular epithelial permeability and keratinization. Enrichment pathway analyses will be performed to better understand these findings and to further identify molecular targets for nutritional or management interventions.

Key Words: dairy cow, digital dermatitis, RNAseq

W6 Transcriptional alterations due to sole ulcer revealed via RNA-seq analysis of corium tissue in lactating dairy cows.

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Ulceration of the sole can damage the corium and affect the keratinization of the hoof tissue. An RNA-seq analysis was performed in corium tissue biopsies to provide a comprehensive transcriptome profile of the hoof. Multiparous Holstein dairy cows were selected based on their condition as clinically healthy (HSU, n = 7), or diagnosed with sole ulcers (SU, n = 7). Lameness was assessed based on a locomotion score (1–5 scale). Hoof hardness was assessed using a durometer (Shore A 0–100HA). Locomotion scores were 1 ± 0 and 2.5 ± 0.5 (mean ± SD) for HSU and SU, respectively. Hoof hardness was 79.8 ± 6.1 and 69.9 ± 4.4 for HSU and SU, respectively. Hoof biopsies were performed according to Osorio et al., 2012; *J Dairy Sci*, 95:6388. All corium samples were immediately flash-frozen in liquid nitrogen. The RNA integrity number for all samples was 8.0 ± 1.0. The RNA samples were sequenced (NGS; Illumina, NovaSeq S4) at the University of Minnesota Genomics Center. Processed reads were aligned to the bovine genome using HISAT2. Differential transcript analysis was performed using a quasi-likelihood test in R-package (EdgeR) and False Discovery Rate < 0.05 correction was applied. Among the 500 differentially expressed genes identified, SU upregulated the expression of genes related to the Toll-like receptor (TLR) pathway including *TLR2*, *5*, and *6* ($P < 0.01$) in comparison to HSU. Genes mediators of pro-inflammatory response *IL1R1*, *IL18R1*, and *IL18RAP* ($P \leq 0.03$) were upregulated in the SU in comparison to HSU. Keratin-associated proteins (KRTAP) including *KRTAP11–1*, *KRTAP15–1*, *KRTAP19–3*, and *KRTAP27–1* were downregulated ($P < 0.002$) in SU than HSU. The greater ($P = 0.005$) expression of *COL6A2* in hoof tissue of SU cows than HSU can be associated with extracellular matrix structural function impacted by sole ulcer. Our preliminary data further confirms that ulcerations can develop inflammatory-like conditions while decreasing keratinization of the hoof. Further analysis of this RNA-seq data can provide novel molecular targets for nutritional interventions to reduce sole ulcer incidence and prevalence in dairy herds.

Key Words: dairy cow, hoof, transcriptome

W7 Case study: Do low-risk cows in a data-driven selective dry-cow program remain low risk at a subsequent dry-off?

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A data-driven, selective dry-cow therapy algorithm has been used often in trials by the authors and has been implemented by US dairy producers to target prudent use of antimicrobials. This case report retrospectively evaluated the recurrence, characteristics, and culture results of cows from a NY dairy that were identified as "low risk" by the algorithm during the 2nd year of using the program. All cows eligible for dry-off had the opportunity to be enrolled from April 2018 to Oct 2018 and again from May 2019 to Aug 2019. "Low risk (LR)" cows (cows that likely will not benefit from antibiotics) were identified as having all of the following: < 200k cells/mL at last test day before dry-off, an average somatic cell count <200k cells/mL on the last 3 test days, no signs of mastitis at dry-off, and no more than 1 clinical mastitis event in the current lactation. Quarter-level milk samples were aseptically retrieved at dry-off during both periods and were submitted for diagnostic aerobic culture and MALDI-TOF following National Mastitis Council guidelines. Of the LR cows (>1st lactation) dried off during the second evaluation period (n = 166), 120 (75%) were identified as being present in the first dry-off period. The distribution by lactation for 2nd, 3rd, and greater than 3rd was 57%, 25%, and 18%. The majority of cows were LR during both periods (n = 101, 84.2%), while the remaining 19 were identified as high risk (HR) during the first period. Of the functional quarters (n = 469) from the 120 cows, bacteriology indicated that 77% were negative at dry-off for both periods. The common remaining culture results were quarters with *Staphylococcus* species (NAS) at the first sampling period and culture negative at the second (9%), contaminated at the first and negative at the second (3%), and culture negative at the first and NAS at the second (3%). For the 19 cows that were HR during the first dry-off period, 13/19 (68%) had a pathogen in at least 1 quarter during the first period. Five of 13 were major mastitis pathogens. This study indicated that LR cows remain consistent in their algorithm assignment over the course of 2 dry-offs and most quarters remain culture negative.

Key Words: selective dry-cow therapy

W8 Dry-off antibiotic use in dairy cattle to cure intramammary infections: A systematic review and network meta-analysis.

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Dry-cow antibiotic therapy is used to both cure existing intramammary infections (IMI) at drying off and to prevent new IMI from occurring during the dry period. IMI increases the risk of clinical mastitis (CM) in the subsequent lactation, therefore, dry-off antibiotics are commonly used during this period to decrease the impact of IMI or CM on milk quality and quantity. The objective of this research was to determine the relative efficacy of dry-off antibiotics labeled for use to cure IMI in dairy cattle through a systematic review and network meta-analysis approach. A systematic search of relevant databases (Medline, CAB Abstracts, Science Citation Index, Conference Proceedings Citation Index – Science, and Agricola), conference proceedings, and FDA summaries was conducted to identify relevant articles. Eligible studies were controlled trials with natural disease exposure evaluating dry-off antibiotic treatment for cure of existing IMI, in comparison to a different antibiotic treatment, a non-antibiotic treatment, or no treatment. From 3749 unique citations identified by the search, there were 149 eligible trials with 236 treatment arms remaining for data extraction, which was completed independently in duplicate. Data from these studies will be synthesized using a network meta-analysis to assess the efficacy of multiple dry-cow antibiotic options through the use of both direct and indirect evidence. If products are equivalent in efficacy, one with a lesser level of importance for human health can be chosen for use by producers. This research will aid veterinarians and dairy producers in making evidence-based decisions concerning antibiotic use and will support the judicious use of antibiotics in dairy cattle.

Key Words: dry cow, mastitis, treatment

W9 Characterization of serine-aspartate rich (Sdr) proteins in *Staphylococcus aureus* isolates from bovine intramammary infections in Canada.

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Many *Staphylococcus aureus* surface proteins represent key elements in the pathogenic process leading to bovine intramammary infection (IMI) and mastitis. We characterized the diversity of serine-aspartate repeat (Sdr) surface proteins found in *S. aureus* strains collected from quarter milk samples in Canada. Strains were selected to represent the main *spa*-types found in Canadian dairy cattle (t529, t267, t359, t605, t13401, t2445). Two groups (t605 and t13401) were strong biofilm producers and *spa* type t2445 was composed of weak biofilm producer strains. The *spa* type t529 was the most prevalent and associated with the presence of the superantigen gene *seg*. Whole-genome sequencing data from the mastitis-associated *S. aureus* strains (36 strains in total) was used to analyze the distribution of 3 specific *sdr* genes (*sdrC, sdrD* and *sdrE*) in the different *spa* groups. SdrC was present in all studied strains except one from the t13401 group. Interestingly, strains from t605 and t529 had the same mutation leading to an early stop codon in *sdrC.* Also, there was a deletion of 330 bases in the *sdrC* gene of t2445 strains (the low-biofilm producers), occurring in a repeated region of the protein rather than in the functional region. SdrE was found in all *spa* groups except for t13401. Surprisingly, an allelic variant of *sdrE* was noticed in t529 isolates. This variant, named bone sialoprotein-binding protein, is characterized by a modified functional domain of the protein and was previously linked to strains leading to bone infections in humans. SdrD was present in 3 *spa* types (t267, t2445 and t359) and its sequence was very similar in all those strains. These results revealed that most *S. aureus* strains from quarter milk samples carry *sdrC* and *sdrE* and that important sequence differences exist between some *spa* groups. However, *sdrD* seems less common. Considering that *S. aureus* can cause variable infection patterns in dairy cattle, understanding the diversity of virulence factors should lead to a better identification of problematic strains and lead to more precise herd management decisions.

Key Words: bovine mastitis, *Staphylococcus aureus*

Breeding and Genetics 1

W10 Genomic predictions for wellness traits in crossbred dairy cattle. D. Gonzalez Pena*, N. Vukasinovic, J. Brooker, C. Przybyla, and S. DeNise, *Zoetis*, *Portage, MI*.

Increase in crossbreeding in US dairy industry has been motivating research on breed-specific parameters. The objective of this study was to evaluate predictivity of ssGBLUP for wellness traits in crossbreed dairy cattle. The traits included were mastitis (MAST), metritis (METR), retained placenta (RETP), displaced abomasum (DA), ketosis (KETO), and lameness (LAME). Genotypes of 44K Holstein x Jersey crossbreds (C) were combined with Holstein (H) and Jersey (J) genotypes in 6 scenarios: 1) C; 2) C + H and J connected through pedigree; 3) C + sires connected through pedigree and phenotypes; 4) C + sires connected through pedigree and phenotypes+ all J; 5) C + H; 6) C + H + J. The number of phenotypes ranged from 3M for KETO to 6M for MAST. For the validation, phenotypes of 960 C were removed and their genomic transmitting abilities (gPTA) were obtained, ranked from the lowest (most desirable) to the highest (least desirable) gPTA, and divided in 3 equally sized groups. Average incidence of a disorder was calculated for each group. Means of gPTA (reliabilities) by scenarios ranged from −0.67 to 0.38 (0.34 to 0.46), −2.13 to −1.03(0.33 to 0.43), −1.41 to −0.55 (0.34 to 0.45), −1.37 to −0.51 (0.27 to 0.40), −1.25 to −0.29(0.27 to 0.39), and −0.22 to 0.23 (0.31 to 0.41) for MAST, METR, RETP, DA, KETO, and LAME, respectively. All traits except DA showed a clear separation among the 3 groups based on average incidence. Scenarios showing the steepest increase in average incidence from the best to the worst group were preferred. Regarding that criterion, scenario 2 was the best for DA, KETO and LAME, whereas the best scenarios for MAST, METR and RETP were 4, 5, and 3, respectively. The difference in average incidence between the worst and the best group in their respective best scenarios was 11.1%, 5.4%, 1.9%, 2.6%., 3.9%, and 6.4% for MAST, METR, RETP, DA, KETO, and LAME, respectively. The lack of agreement between scenarios might be reflecting different connectivity among the genotypes and phenotypes of animals included in the analysis of each trait.

Key Words: crossbred, wellness traits

W11 Genomic predictions for crossbred animals with single-step genomic BLUP. D. Gonzalez Pena*[1], N. Vukasinovic[1], J. Brooker[1], C. Przybyla[1], Y. Steyn[2], and S. DeNise[1], [1]*Zoetis*, *Portage, MI*, [2]*University of Georgia*, *Athens, GA*.

Crossbreeding in US dairy herds is increasing, motivating research on breed-specific parameters. This study investigates the use of ssGBLUP for generating genomic estimated breeding values (gEBV) of crossbreed dairy animals for milk (MY), fat (FY), and protein (PY) yield, daughter pregnancy rate (DPR), somatic cell score (SCS), and productive life (PL) under different scenarios. Genotypes from 375K Holsteins (H), 60K Jerseys (J), and 21K HxJ crosses (C) were available. The C genotypes were combined in 6 scenarios: S1) only C; S2) C + H and J connected through pedigree; S3) C + sires connected through pedigree and phenotypes; S4) C + sires connected through pedigree and phenotypes + all J; S5) C + all H; S6) C + all H + all J. All scenarios used the same phenotypes and a pedigree depth of 20 generations. Number of records was 13,636,724 for MY, FY, PY, and SCC; 9,531,611 for DPR, and 4,100,343 for PL. The performance of each scenario was validated using 1,987 C animals with phenotypes and genotypes, with phenotypes being removed from the analysis. The predictivity was defined as the correlation (r) between gEBV and phenotypes adjusted for the fixed effects in the model. The inflation of gEBV was measured by the regression coefficient (b) of the adjusted phenotype on the gEBV. For MY, S2 had the highest (r = 0.42) and S6 had the lowest predictivity (r = 0.30). For all other traits, S4 performed best with r = 0.31, 0.27, 0.14, and 0.22 for FY, PY, DPR, and SCS, respectively. Both S4 and S6 had r = 0.12 for PL. The worst scenarios for FY, PY, DPR, PL, and SCS were S6 (r = 0.21), S6 (r = 0.15), S5 (r = 0.09), S3 (r = 0.02), and both S1 and S6 (r = 0.17), respectively. Regarding gEBV inflation, S2 was the best for MY (b = 0.89), whereas S4 was the best for all other traits (b = 0.87, 0.73, 1.04, 1.0, 0.78 for FY, PY, DPR, SCS, and PL, respectively). Limited predictivity of only C genotypes and the benefits of including related purebred animals are visible. The number of purebred genotypes in the analysis had an impact on the crossbred gEBV under ssGBLUP, which could be associated with the proportion of chromosomal segments shared among crossbred and purebred animals.

Key Words: crossbred, genomic

W12 Genetic parameters of horn fly resistance in pastured Holstein cattle. B. L. Basiel*[1], L. C. Hardie[1], B. J. Heins[2], and C. D. Dechow[1], [1]*Pennsylvania State University*, *University Park, PA*, [2]*University of Minnesota*, *St. Paul, MN*.

Horn flies (*Haematobia irritans irritans*) contribute to major economic losses of pastured cattle operations. Infestation can cause reduced milk yield and spread disease-causing pathogens which further reduce yield and incur health expenses. Most fly control methods require synthetic chemicals that cannot be used on organic operations. The objective of this research was to determine if natural resistance to horn flies is a heritable trait that can be incorporated into a traditional breeding program. Observations of fly load were recorded from pastured Holstein cows on 13 organic dairies across the United States. Fly load was determined using a 0 to 4 scale based on fly coverage from chine to loin on one side of the body; a score of 0 indicated few to no flies and a score of 4 indicated high infestation. There were 1,823 records of fly load from 1,265 Holstein sired cows included in the analysis. The scoring system was validated by counting flies from photographs taken at the time of scoring on a subset of observations (n = 290). Contemporaries that left the herd before scoring were accounted for by merging 160,740 observations of stayability with fly score records. The rank correlation between the subjective fly score and the objective count was 0.80 ($P < 0.0001$). Genetic parameters were determined using mixed models in ASReml 4.1 that included fixed effects of percent pedigree completeness (50% to 100%) to account for variation contributed by unknown breeds in the pedigree, herd-year-season of calving, days in milk, scorer, date, and age at calving with parity group (1, 2, ≥ 3) for fly score and count; stayability included fixed effects of parity (1 to 5), herd-year-season of birth, and pedigree completeness. Random effects included animal and permanent environment for all traits. The heritability of fly score was 0.09 ± 0.05 with a repeatability of 0.21 ± 0.04. The genetic correlation between fly load and stayability was not significant. The magnitude of heritable variation in fly infestation is similar to other traits included in selection programs, suggesting that we can select for resistance to horn flies.

Key Words: fly, organic, heritability

W13 Associations between predictions of Lifetime Net Merit and profitability of dairy cows. G. L. Pezzella*[1], C. D. Dechow[2], and A. De Vries[1], [1]*University of Florida*, *Gainesville, FL*, [2]*The Pennsylvania State University*, *University Park, PA*.

The objective of this study was to determine the associations between estimates of Lifetime Net Merit (NM$) that were derived independently of the cow's own performance, and observed profitability of dairy cows. The Pennsylvania State University provided daily BW and daily milk production data (yields, fat, and protein), birth dates, calving dates, dry-off dates, and disease treatment data for 2,305 cows in their herd from 1998 to 2017. Python and Microsoft Excel were used to calculate the total milk revenue, health and feed costs, and net profit per cow per lactation. The health events in the analysis include displaced abomasum, mastitis, ketosis, metritis, milk fever, and retained placenta with their costs taken from the literature. Replacement and other costs were not included. Profit was measured as total lifetime profit and standardized lifetime profit calculated as profit per day x 305 × 2.78. Genetic estimates of NM$ included Direct Genomic Values (DGV) from the April 2018 genetic evaluation for 725 cows and Predicted Transmitting Ability (PTA*) values calculated as sire + 1/2 maternal grandsire + 1/4 maternal great-grandsire PTA from the December 2019 genetic evaluation. Therefore cows' own performance data had little effect on genetic estimates. Simple linear regression was used to calculate slopes (b) and R^2. Results showed a positive association between PTA*(y) and DGV(x) ($n = 671$, $b = 1.544$, $R^2 = 0.700$) where the expected value was $b = 1.75$. The DGV predicted total lifetime profit ($n = 532$, $b = 1.599$, $R^2 = 0.062$) and standardized lifetime profit ($n = 532$, $b = 2.139$, $R^2 = 0.124$) where the expectation was $b = 2$. The PTA* predicted total lifetime profit ($n = 1{,}109$, $b = 1.740$, $R^2 = 0.059$) and standardized lifetime profit ($n = 1{,}109$, $b = 1.180$, $R^2 = 0.128$) where the expectation was $b = 1.14$. These findings confirm that predictions of genetic merit of profit can be observed in differences in real profitability of dairy cows. The association was strongest for standardized lifetime profit, which implies that the opportunity cost of delayed replacement must be considered.

Key Words: profitability, genetics, selection index

W14 Predictions of daily total sperm production of Holstein and Jersey bulls using health and management data.

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Selection of elite young bulls using genomic data has shortened the generation interval and increased pressure to collect large quantities of semen at an early age. The objective of this study was to develop a prediction model for daily total sperm (TS) production from collection history, health status, and management factors. Data were obtained from a commercial AI company and included 47,199 daily collection records from 1,118 Holstein and Jersey bulls between 10 and 30 mo of age. Potential explanatory variables included: year and season of collection, barn location at collection, days since last collection, number of collections per week, number of ejaculates per collection day, breed, scrotal circumference (SC) at 10–11 mo of age, prior health events (binary incidences in last 1, 3, or 6 mo), and age at arrival, first collection, and current collection. Four analyses were carried out based on availability of prior TS data, each split into 75% training data and 25% testing data: no prior collection requirement (Lag0; $n = 47{,}199$), at least one prior collection (Lag1, $n = 46{,}081$), at least 2 prior collections (Lag2, $n = 44{,}987$), and at least 3 prior collections (Lag3, $n = 43{,}918$). Adjusted R-squared for linear regression models (R v3.5.1) ranged from 0.42 for Lag0 to 0.54 for Lag3. The most significant explanatory variables were collection age, days since last collection, number of collections per week, and number of ejaculates per collection day ($P < 2 \times 10^{-16}$). Inclusion of previous TS values in the lag analyses (Lag1, Lag2, Lag3) was highly significant ($P < 2 \times 10^{-16}$), with TS second prior having most influence. In all 4 analyses, SC maximized TS when SC is 32.5 to 34.5 cm ($P < 6.12 \times 10^{-7}$) and minimized when SC <28.5 cm ($P < 0.05$). Age at first collection and incidence of hoof trimming (last 6 mo), dehorning, and respiratory system (last 3 mo) events were significant ($P < 0.05$) in all 4 analyses. Jerseys ($P < 4.62 \times 10^{-14}$) produced more TS than Holsteins.

Key Words: sperm production, management factors, prediction

W15 A recessive *IL17RA* frameshift variant in Holstein cattle with psoriasis-like skin alterations and immunodeficiency.

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Skin lesions and dermatoses in cattle are often associated with infections. Inherited skin disorders or genodermatoses are primary congenital disorders or dermatoses that are associated with nutritional deficiencies, e.g., bovine hereditary zinc deficiency or zinc-deficiency-like syndrome. We observed 3 German Holstein cases with skin lesions that resemble zinc deficiency-like syndrome. Clinical blood analysis showed leukocytosis with neutrophilia indicating inflammation and pathology revealed severe ulcerative dermatitis with hyperkeratosis, alopecia furunculosis and subcutaneous abscess formation. The origin of all affected calves from a single sire and the same maternal grand sire let to the assumption of a potential recessive defect. Due to the suspected genetic background, whole-genome sequencing of 2 of the affected Holstein calves was performed. Genetic analyses delivered several variants shared by both affected animals, however, comparison with the current 1000 Bull Genomes variant catalog resulted in a single protein-changing variant private to the 2 cases. This single-nucleotide deletion in exon 3 of *IL17RA* on bovine chromosome 5 was predicted to have a deleterious impact on the encoded protein due to a frameshift. Healthy mothers of affected calves as well as some other relatives were determined to be heterozygous for this most likely pathogenic variant by Sanger sequencing, confirming the assumed autosomal recessive inheritance. In man, rare diseases are associated with *IL17RA* include immunodeficiency 51 and chronic mucocutaneous candidiasis. The frequency of the recessive loss-of-function variant in *IL17RA* needs to be analyzed within the German and global Holstein population.

Key Words: monogenic, genodermatoses, rare disease

W16 Beyond studying genetic diversity: How can pedigree and genomic data help us assign individuals to breeds?

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Determining to which breed an individual belongs is not always an unbiased choice. Definition of breeds is not always clear and takes some subjective elements into account (e.g., phenotypes or administrative rules). Moreover, insufficient pedigree deepness worsens this issue. This explains the need for development of breed assignment tools and their routine use. This kind of tools supposes a known "Reference population" containing maximum genetic diversity of the breed considered. Moreover, "Candidate individuals" have to be close enough to this "Reference population" to allow correct individual assignment. Tools based on classification methods allow breed assignment and subsequently subsidy payment schemes in Wallonia (Southern Belgium) and Luxembourg. Currently, a principal component analysis (PCA) based on genotypes is used as a routine to determine if individuals belong to 2 local dual-purpose cattle breeds (i.e., East Belgian Red and White, Belgium, and Ösling Red Pied, Luxembourg). This analysis relies on the position of individuals on the PCA compared with those of reference individuals from different breeds (East Belgian Red and White and Ösling Red Pied but also "sister breeds" and (Red-)Holstein). However, the continuum of Red-Pied breeds in Western Europe makes it difficult to choose to which breed the animal belongs. One example is the overlapping observed on the PCA between East Belgian Red and White and Ösling Red Pied. One solution is maybe to include these animals in both herdbooks. This would allow exchange program between so closely related breeds. Furthermore, the question arises to what extent peripherical individuals, but potentially phenotypically interesting, should be included, as they could provide more diversity to the current gene pool. In these cases, the study of different parameters that inform us about inbreeding (e.g., runs of homozygosity or effective population size) and admixture within breeds or differentiation levels (e.g., fixation index) between breeds, can help to assign individuals to breeds.

Key Words: breed assignment, genetic diversity, genotypes

W17 Genetic and environmental changes in dairy traits revealed from a genetic base update. H. D. Norman*[1], P. M. VanRaden[2], and J. W. Dürr[1], [1]*Council on Dairy Cattle Breeding*, *Bowie, MD*, [2]*Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA*, *Beltsville, MD*.

The genetic bases to which (most) dairy traits are expressed in the United States has been updated every 5 years since 1980. For a base change, the average predicted transmitting abilities (PTA) for cows born in the designated year is subtracted from the current PTAs for all animals; i.e., it's the gain (or loss) in PTAs for animals across the 5-year period. The rationale for changing the base is to make users of genetic evaluations aware the standards set for genetic selection in the past may no longer meet the quality needed to remain competitive. Eighty-one of the 102 breed-traits for yield and fitness traits showed favorable gains. The base change for Holsteins and Jerseys will reduce PTA milk by about 223 and 238 kg., respectively. PTAs for fat and protein will be adjusted down by about 8 to 11 kg. Changes in PTAs for somatic cell score (SCS) will be small for all breeds except Holsteins (0.08). PTAs for productive life will be reduced by 0.6 to 1.9 mo for 4 breeds. Changes among breeds in fertility trait were unexpected; only Holstein improved in the 3 traits. The PTAs for cow livability improved for 3 of the 6 breeds (0.74 mo for Holsteins). Holstein resistance against diseases improved for 5 of the 6 traits. The genomic revolution initiated in 2008 increased the rate of genetic improvement, primarily due to a reduction in the generation interval. Improvement in Brown Swiss, Holsteins and Jerseys were greater for milk traits (7 to 64%) than for the previous base change. The Guernseys, Holsteins, and Jerseys showed larger gains (43 to 100%) in the lifetime merit indexes than during the previous 5-years. Results show how much of the changes in phenotypes in dairy traits that had evaluation initiated since 2008 is attributed to genetics and environment. The genetic contribution accounted for 45%, but was greater for the 3 traits with the largest emphasis in net merit dollars (averaged 69%) and for the most populous breed, Holsteins (71%). These changes in productivity should help eliminate world hunger and reduce greenhouse gas emissions from requiring fewer animals.

Key Words: genetic base, national evaluation, predicted transmitting ability

W18 Predicted feed efficiency index applied to Italian Holstein Friesian cattle population. F. Omodei Zorini*[1], R. Finocchiaro[2], G. Savoini[1], G. Invernizzi[1], and M. Cassandro[3], [1]*Department of Health, Animal Science and Food Safety 'Carlo Cantoni', University of Milan*, *Milan, Italy*, [2]*Italian Holstein and Jersey Association (ANAFIJ), Research and Development Office*, *Cremona, Italy*, [3]*Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padua*, *Padua, Italy*.

Selection for feed efficiency is important to improve the economic and environmental sustainability of the dairy cattle industry. Many countries and international research projects are working on the possibility to select animals that efficiently transform feed into milk products. Feed efficiency matters on farms because it has a major influence on farm profitability and environmental stewardship in the dairy industry. The aim of this study was to describe a new selection index adopted by ANAFIJ for improving feed efficiency using data recorded by the official recording system. Predicted dry matter intake (pDMI) was derived from milk yield, fat content, and cow estimated BW. Energy milk content (FPCM) was derived from milk yield corrected for fat, protein and lactose content using the Sjaunja equation. Therefore, the predicted feed efficiency was estimated as ratio between FPCM and pDMI. Average predicted feed efficiency was equal to 1.26 ± 0.18 with heritability equal to 0.32. Predicted feed efficiency index (pFE), traditional and genomic, has been implemented in the Italian Holstein Friesian evaluation system. Results suggest that pFE may be a new breeding objective for Italian Friesian. At this time the trait is derived by exploiting the national recording system, without any new additional trait. The official selection index (PFT), in use since 2002, is positively correlated with pFE meaning that breeders have already started selecting for more efficient animals. However, the introduction of a the pFE index, as a direct "tool" will improve the positive feed efficiency trend. This approach will permit the Holstein Friesian breeders in Italy to improve feed efficiency, without increasing costs of recording system. However, to avoid the risk to select animals with an excessive negative energy balance after calving, it should be useful to include in the pFE a correction for BCS and reproductive performances. In the meanwhile, to increase the accuracy of the predicted phenotype, an Italian consortium is creating a consistent phenotypic critical mass of individual data for DMI in cows, heifers and young bulls.

Key Words: feed efficiency, Italian Holstein Friesian dairy cattle, dry matter intake

W19 Quality control to improve properties of sequence genotypes from different sources. D. J. Null*, J. B. Cole, A. Al-Khudhair, and P. M. VanRaden, *USDA Animal Genomics and Improvement Laboratory*, *Beltsville, MD*.

Sequence genotypes from run7 of the 1000 Bull Genomes Project, high-density array genotypes for many of the same bulls, and additional sequence data were examined to determine optimal editing strategies. The 3,093 sequenced animals in the run7 *Bos taurus* analysis included 928 Holsteins, 175 Brown Swiss, 156 Ayrshires/Red Dairy Cattle, 105 Jerseys, 51 Montbéliardes, 22 Normandes, and 20 Guernseys; 1,429 were selected as animals of interest after editing or removing bulls with low coverage; incorrect identification, breed, or pedigree; duplicate sequence genotypes; or sequence genotypes that were inconsistent with chip genotypes. An additional 241 bulls had sequence variants identified locally by SAMtools rather than globally by GATK now used in run7. For chromosome 29 as an example, the raw global analysis identified 149,684 variants, and the local data identified 99,600; surprisingly, the overlap was only 48,266 variants. Thus, half of the variants in local data were not in the global data, which were expected to be a superset. Known lethal recessive alleles affecting fertility were present and retained. For quality control, array genotypes from the Council on Dairy Cattle Breeding (Bowie, MD) database included either 79,294 SNP from routine predictions or 643,059 SNP from imputed high-density genotypes. Sequence genotypes for 534 of the run7 animals had matching array genotypes from national data. Concordance of genotypes was better with run7 raw data (98.6% for 69,433 matching SNP) than from the run7 Beagle-imputed subset (98.0% for 61,299 SNP). After excluding multiallelic variants, which were 9% of the run7 raw variants, 48,056,551 variants were polymorphic in the 1,429 dairy animals and included 11% insertions and 4% deletions. Genotypes were then edited for missing rate, parent-progeny conflicts, excess heterozygotes, and minor allele frequency of > 1% in at least 1 breed. After removing loci in a few potentially mismapped regions of the ARS-UCD1 reference map, an edited total of 6,735,530 loci were available to impute genotypes for other animals and investigate phenotypic effects.

Key Words: variant calling, genotype concordance, sequence variant

W20 Genome changes due to selection in US dairy cattle. E. Freebern*[1], J. Jiang[2], J. B. Cole[3], P. M. VanRaden[3], and L. Ma[1], [1]*University of Maryland*, *College Park, MD*, [2]*North Carolina State University*, *Raleigh, NC*, [3]*Animal Genomics and Improvement Laboratory*, *Beltsville, MD*.

Genetic and genomic selection in the US dairy population has achieved successful phenotypic improvement across a comprehensive set of economically important traits that involve production, reproduction, health, and body conformation. While contemporary cows differ phenotypically from their ancestors hundreds of years ago, the changes in the genome, especially those due to selection, remain to be discovered. The aim of this study is to investigate genome-wide and region-specific changes in the US Holstein-Friesian (HF) cattle population between the years of 1950 to 2015. Using the US dairy cooperator' phenotypic and genomic databases hosted at CDCB, we first extracted genotype and phenotype (PTA) data of 27,000 reference bulls and performed GWAS analyses to identify candidate QTLs. We then divided the 27,000 Holstein bulls into 9 bins based on birth year, before 1980, 1980–1990, 1990–1995, 1995–2000, 2000–2002, 2002–2005, 2005–2007, 2007–2010, and after 2010. The allele frequency changes between the 2 extreme time periods were calculated to capture the difference between the earliest and most recent populations. Finally, the genomic regions with the largest allele frequency changes were compared against the QTL regions identified in GWAS analyses. To identify true genome changes due to selection from those due to random genetic drift, we implemented a gene dropping simulation approach with real pedigree and calculated thresholds of allele frequency change. The process was executed by running a simulation program in Python, which will visualize systemic changes over individual SNPs and compare them to a distribution under pure genetic drift. Observation of changes above the 99.9% threshold on the distribution may be indicative of selection and affecting dairy traits. From this evaluation of genome-wide and region-specific changes due to selection, we will identify candidate QTL regions under selection and that are associated with economically important traits in the US dairy population.

Key Words: genomic selection, GWAS, allele frequency changes

W21 Changes in early milk composition has subsequent effects on microbial composition of the rumen. A. Nin-Velez*[1], J. Duncan[1], H. Cunningham-Hollinger[2], K. Austin[2], K. Cammack[2], W. Lamberson[3], and R. Cockrum[1], [1]*Virginia Polytechnic Institute and State University*, *Blacksburg, VA*, [2]*University of Wyoming*, *Laramie, WY*, [3]*University of Missouri*, *Columbia, MO*.

Components within early milk provide continual inoculation of microbial species within the calf gut. The gut microbiome stimulates development of epithelial tissue and immune cell differentiation. Though limited research suggests milk components are influenced by mode of birth, it is unknown how the maternal environment influences the composition of early milk. We hypothesized that mode of birth would impact early milk composition, and, in turn, influence the microbial taxa in the calf gut. Early milk samples were collected from Charolaise (n = 35) and Angus (n = 34) dams on d 1, 3, and 28 postpartum who underwent vaginal (VD) or cesarean (CD) delivery. Components and fatty acid composition were determined for milk samples. Calf rumen fluid samples were obtained for d 1, 3, and 28 and were used for metagenomic sequencing. Samples were run on the Illumina HiSeq 2500 platform as paired end reads. Mode of birth was regressed on milk composition for each day using the GLM procedure in SAS. The GENMOD procedure was conducted with milk components being regressed against microbial counts. Results indicated that VD dams were more likely to have increased protein, solid nonfat (SNF), and lactose ($P = 0.001$) on d 1 and 3, but decreased urea concentrations ($P < 0.001$). Similarly, short, medium, and long-chain fatty acids were increased ($P = 0.051$) in VD d 3 milk. True protein elicited a decrease ($P = 0.033$) in rumen fluid *Actinobacteria* and *Proteobacteria*; whereas, both SNF and lactose were associated with an increased ($P = 0.049$) response in d 1 milk. Based on these results, we suggest that mode of birth influences protein concentrations in early milk, and even though we can see some changes to microbial abundance the overall dynamics of the calf rumen microbiome are relatively stable in response to changes in milk composition.

W22 Relationship between milk production and indicator traits of robustness in first-parity Holstein cows. C. H. F. Zago Dias*[1], L. El Faro[2], M. L. Santana Jr[3], R. A. Teixeira[1], A. A. Valotto[4], and L. T. Dias[1], [1]*Universidade Federal do Paraná*, *Curitiba, Paraná, Brazil*, [2]*Instituto de Zootecnia*, *Sertãozinho, São Paulo, Brazil*, [3]*Universidade Federal de Rondonópolis*, *Rondonópolis, Mato Grosso, Brazil*, [4]*Associação Paranaense dos Criadores de Bovino da Raça Holandesa*, *Curitiba, Paraná, Brazil*.

The current major challenges of dairy cattle improvement are to select animals with a greater capacity to overcome the metabolic disorders in postpartum. This study aimed to evaluate the association of the type traits angularity (ANG) and body conditions score (BCS) with test-day milk yield (TDMY) to identify more robust animals. In this study, 301,301 TDMY records from 33,543 first-parity Holstein cows, which belonged to 146 herds in the state of Paraná, Brazil, were analyzed. Angularity and BCS were classified at an average 88 d of lactation. A 2-trait random regression model between TDMY and the type traits was applied. The model included the fixed effects of contemporary group (herd, test-day year and month) and the linear and quadratic effects of cow age at calving and interval between classification date and calving (only for ANG and BCS) as covariates. The fixed lactation curve for TDMY was modeled using quadratic Legendre polynomials. For TDMY, additive genetic and animal permanent environmental effects were modeled as random effects using cubic Legendre polynomials, in addition to the residual effect. For ANG and BCS, only the additive genetic effects and residual were included as random effects. The (co)variance components were estimated by Bayesian inference using the GIBBS2F90 program (Misztal et al., 2002). The heritability for TDMY ranged from 0.22 to 0.36, with higher estimates close to peak yield. The heritability estimates for ANG and BCS were 0.17 ± 0.03 and 0.19 ± 0.02, respectively. Genetic correlations between TDMY and ANG or BCS were negative and antagonistic throughout lactation, with stronger correlations between TDMY and BCS at 30 d of lactation (−0.72) and between TDMY and ANG from 90 to 120 d (around −0.86). A higher genetic association was observed between BCS and TDMY at the beginning of lactation, a critical period when cows are in negative energy balance, while ANG showed a higher genetic association close to peak yield. Thus, the type traits studied may be used as auxiliary measures to identify more robust animals considering the different lactation periods.

Key Words: random regression model, transition period, type traits

W23 Evaluating the correlation of digital dermatitis infection and genetic variants to predict gene resistance in Holstein cattle. G. M. Canny*[1], C. L. Hendley[1], W. B. Smith[1], B. W. Jones[1,2], and J. N. Waddell[1], [1]*Tarleton State University*, *Stephenville, TX*, [2]*Texas A&M AgriLife Research*, *Stephenville, TX*.

Digital dermatitis (DD) is a contagious bacterial hoof infection. Determining genetic resistance of DD will allow dairy producers to select for this trait, therefore reducing lameness and treatment cost. This study evaluated the correlation of infection and genetic variants to predict genetic resistance of DD resistance in Holstein cattle on a commercial dairy farm in central Texas. Lactating dairy cattle were scored on an M0 (early lesions) to M4 (chronic lesions) scale. Blood samples were collected on lactating cows (n = 45) from the coccygeal vein for genomic DNA isolation. Cows were identified as having a lesion (n = 35) and not having a lesion (n = 10). Four previously reported SNPs were tested on genomic DNA to determine correlation to DD resistance by qPCR melt curve analysis. SNPs in the 3′UTR of C94T, A22DG, interleukin-8 (*IL8*) and exon 3 of toll-like receptor 4 (*TLR4*) were analyzed by Chi-Square in SAS (Version 9.4 SAS Institute, Inc., Cary, NC) to determine the frequencies of different genotypes present among the resistant and affected animals. One SNP in *Bta-IL8* resulted in a significant chi-square analysis ($P = 0.03$). These results indicate a genetic correlation to resistance to DD within the *Bta-IL8* gene. This qPCR melt curve analysis can lead to a fast, affordable genotyping assay to select for cows genetically resistant to this costly infection.

Key Words: digital dermatitis, gene resistance, interleukin-8

W24 Predicting feed intake and heath events using sensor data in lactating Holstein cows.

C. J. Siberski*[1], M. S. Mayes[1], P. J. Gorden[2], A. Copeland[2], M. Healey[1], B. M. Goetz[1], H. Beiki[1], L. M. Kramer[1], L. H. Baumgard[1], P. Dixon[3], and J. E. Koltes[1], [1]*Department of Animal Science, Iowa State University*, *Ames, IA*, [2]*Veterinary Diagnostic & Production Animal Medicine, Iowa State University*, *Ames, IA*, [3]*Department of Statistics, Iowa State University*, *Ames, IA*.

Feed intake and cow health are highly interrelated, and impact dairy sustainability. The objective of this study was to evaluate the utility of wearable sensor data to predict feed intake and health events [n = 107 Holstein cows; parity 1–4; day in milk (DIM) 50–270; season = summer (n = 47) and fall (n = 60)]. Three automated sensors were evaluated: 2 ear tags (n = 107 and 41) and a rumen bolus (n = 60). Sensors recorded animal activity, ear temperature, rumination, rumen temperature and pH. Additional traits collected included milk yield and components (fat, protein, and lactose), BW, BCS, and health events from veterinary records. Temperature-humidity index (THI) was calculated from a local weather station located near the dairy. Adjusted daily dry matter intake (ADMI) was calculated utilizing PROC GLIMMIX in SAS 9.4, where DMI was adjusted for milk yield and components, $BW^{0.75}$, DIM, parity and contemporary group. Missing data were imputed utilizing the mice package in R. Prediction was conducted using the random forest algorithm (R-caret package). Feed intake (ADMI) models included sensor, health, BCS and THI. Health models included these variables plus contemporary group, DIM, $BW^{0.75}$, milk components and yield, parity and ADMI. Models with and without cow were considered. Leave-one-out validation was utilized, in which all records across all DIM from each cow were removed. The caret package also applied a 10-fold cross-validation within each leave-one-out data set. The average coefficient of determination for prediction of ADMI was 0.19–0.23 depending on the sensor measures included. Temperature measurements appeared to have the highest variable importance values for ADMI. The average accuracy of predicting health events was 0.94–0.96 (kappa values 0.72–0.82) for all sensors. For health events, activity and ear temperature had the highest variable importance values for sensor measures. Although validation is needed, sensor measures may be useful in commercial settings to detect health events or fluctuations in feed intake.

Key Words: feed intake, health events, prediction

W25 Can metafounders improve inbreeding estimation?

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In sheep, there are considerable difficulties to control pedigrees because of the use of natural mating. This incomplete pedigree information is typically 20% of unknown sire or dam, and inbreeding tends to be underestimated. The concept of metafounder deals with important issues in animal genetic evaluation. Metafounders allow to accommodate and conciliate pedigree and genomic relationships. Inclusion of metafounder relationships also compensates for missing pedigrees, resulting in inbred animals born from unknown parents. Thus, if all animals have estimates of inbreeding, regardless of pedigree completion, more accurate estimates of inbreeding depression could be obtained. The goal of this study is to test the usefulness of metafounder relationships in the estimation of inbreeding depression. Inbreeding coefficients were computed with pedigree and/or markers by considering or not metafounders in Basco-Béarnaise rams. Data comprised 16,196 phenotypic records (semen traits) for 620 rams, 533 of them genotyped with 36,464 SNPs (Illumina OvineSNP50 BeadChip). There were 8,266 animals in the pedigree including these rams and 5 generations of ancestors. Inbreeding coefficients were estimated using 4 methods. The first method used the traditional pedigree-based inbreeding. The second used pedigree-based inbreeding but accounting for nonzero relationships for unknown parents. The third method used the metafounder relationships in pedigree-based inbreeding. The fourth method calculates inbreeding from a combined relationship matrix using pedigree and genotypes with metafounder relationships. Inbreeding depression was estimated as regression on inbreeding coefficient in an animal model including environmental effects, permanent environment and breeding values. These were modeled with pedigree in models 1 and 2, pedigree + metafounders in model 3 and Single-Step with metafounders in model 4. Models 3 and 4 (with metafounders) show that motility was unfavorably impacted (significant estimate of inbreeding depression) by inbreeding in Basco-Béarnaise rams. Metafounders allows more accurate inbreeding depression estimations in motility. Thus, this approach compensates missing pedigree information.

Key Words: inbreeding, genomic, depression

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W26 Comprehensive multi-day and cross-facility evaluation of the industrial Cheddar cheese production environment microbiome. J. Johnson*, B. Selover, C. Curtin, and J. Waite-Cusic, *Oregon State University*, *Corvallis, OR*.

Industrial Cheddar cheese manufacturing has been accomplished through the engineering of equipment and design of manufacturing plants that are intended to improve production efficiency and minimize finished product quality variability. One aspect of cheese quality variability that remains uncharacterized is the microbial variability in cheese production environments within the context of production schedules. Our aim was to capture this variability by monitoring daily and facility-specific changes in microbial communities throughout the cheese production process. Samples of in-process product (milk and cheese curd) and environmental swabs from the draining-matting conveyor (DMC) and salter belts were collected from 3 Cheddar cheese production facilities over 3 consecutive days of production at the beginning, middle, and end of the production day. Diversity of the bacterial community, or microbiome, was determined using high throughput 16S rRNA sequencing. Microbial communities were compared at 3 levels - between replicates, between days at each facility, and between facilities. The extent of facility-to-facility or day-to-day variability differed between sampling locations, with facility differences being greater than daily differences in only 2 of the 9 sample locations considered. The relative abundance of dominant and subdominant taxa was also observed to change considerably between days, further illustrating the importance of repeated sampling in microbiome-based studies. Collectively, this study represents the most thorough investigation to date into the variability of microbial communities within the cheese production environment. These findings will serve as a foundation to support experimental design and sampling strategies for further investigations of the cheese facility microbiome and its impact on cheese quality.

Key Words: cheese, facility, microbiome

W27 Inhibition of *Listeria monocytogenes* in queso fresco by topical application of lactose oxidase. K. Jencarelli*, S. Kozak, M. Lawton, and S. Alcaine, *Cornell University*, *Ithaca, NY*.

Listeria monocytogenes is of major concern in dairy products, especially in fresh cheeses that have high water activity and moisture content, and near-neutral pH. Consumer trends in clean-label foods necessitates the development of strategies to combat pathogens without traditional preservatives. The enzyme lactose oxidase (LO) represents a potential clean-label antimicrobial in dairy products. LO oxidizes lactose to lactobionic acid and produces the antimicrobial hydrogen peroxide. The objective of this research was to determine the efficacy of LO to inhibit *L. monocytogenes* growth in queso fresco. Since cheese has a reduced level of lactose, a 16% lactose solution was mixed 50/50 with LO before its application to the cheese to ensure adequate substrate levels and produce an antimicrobial effect. A 100 µL aliquot of LO was added at varying concentrations (0.012 g/L, 0.12 g/L, 1.2 g/L and 12 g/L) to 5 ± 0.5g of commercial queso fresco inoculated with 2 log cfu/g of *L. monocytogenes*. The inoculum was allowed to attach for 30 min before LO treatments. Two positive controls, inoculated cheese with and without lactose addition, and a negative, uninoculated, control were included. Cheese was stored at 6°C and enumerated on modified oxford agar on d 0, 2, 4, 7, 14, and 21 post-inoculation. An ANOVA and Tukey tests were used to compare differences in *L. monocytogenes* growth among treatments and the positive control. By d 21, *L. monocytogenes* on the positive control reached 7.39 log cfu/g. There was a significant reduction of *L. monocytogenes* growth in both the 12 g/L and 1.2 g/L LO treatments. The level of *L. monocytogenes* in the 12 g/L treatment remained below the detection limit of 2 log cfu/g, while the level in the 1.2 g/L treatment reached 3.04 log cfu/g ($P < 0.001$). *L. monocytogenes* growth in the lactose control did not differ from the positive control without lactose ($P = 0.899$). This study shows that LO is effective in inhibiting the growth of *L. monocytogenes* in the fresh cheese queso fresco at concentrations 12 g/L and 1.2 g/L. Future research will be conducted to study potential sensorial effects of LO in cheese.

Key Words: *Listeria*, queso fresco

W28 Is the presence of biogenic amines a cause of slits and cracks in cheese? I. Panguripan*[1], R. A. Ibanez[2], K. Houck[2], S. Govindasamy-Lucey[2], M. E. Johnson[2], and J. A. Lucey[1,2], [1]*University of Wisconsin-Madison, Department of Food Science*, *Madison, WI*, [2]*University of Wisconsin-Madison, Center for Dairy Research*, *Madison, WI*.

Undesired late gas formation (LGF) in cheese is associated with the presence of slits and cracks. This defect leads to economic losses due to rejection from consumers. A possible source of LGF is attributed to heterofermentative non-starter lactic acid bacteria (NSLAB) that may release CO_2 into the cheese environment. Certain NSLAB have the ability to metabolize amino acids and form biogenic amines (BA) and CO_2. BA are considered undesired compounds in fermented dairy products since they are potentially toxic at high concentrations. In a previous work, our group isolated BA producing bacteria from cheeses with slits and cracks, hence we hypothesized that one of the causes of LGF in cheese is associated with the presence of BA. Therefore, the objectives of this study were to determine the concentration of BA in commercial cheeses with LGF and estimate the concentration of CO_2 based on the levels of BA. Thirteen commercial samples with slits and cracks (Cheddar, 6; Provolone, 1; Parmesan, 1; Romano, 1; Farmers, 1; and block Gouda, 2) were used in this study. Type and concentration of BA in cheese samples were determined by reversed-phase high performance liquid chromatography using a dansyl-chloride derivatization method. The total concentration of BA was used to calculate theoretical concentration of CO_2 in cheeses. Cheese extracts were prepared and inoculated in Durham tubes with broth containing only amino acids as restrictive media to visually observe CO_2 production ability. Tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine and spermidine were detected at varying levels in all cheeses. Levels of total BA and theoretical levels of CO_2 in cheeses ranged between 4 - 2500 mg/kg and 0.02 - 25 mmol/kg respectively. Principal component analysis grouped cheeses based on levels and type of BA, which were also correlated with pH values, suggesting that BA may be responsible for the occurrence of slits and cracks in some cheese samples. Further studies will be necessary to isolate and identify the bacteria involved in BA synthesis and elucidate other sources causing LGF.

Key Words: biogenic amines, late gas formation in cheese, cheese defects

W29 Survivability of *Salmonella* pathogens in powder goat milk stored under refrigeration and room temperatures for 6 months. R. Paswan*[1], A. Mishra[2], and Y. W. Park[1], [1]*Fort Valley State University*, *Fort Valley, GA*, [2]*University of Georgia*, *Athens, GA*.

Powder milk has the advantage of controlling the growth of pathogenic and spoilage microorganisms due to its low water activity (a_w), while a dry food often can pose a possible food safety risk due to the survival of pathogens in the product. The objective of this study was to investigate the survivability of *Salmonella* pathogens in powder goat milk (PGM) under different storage conditions. The study was conducted in a 3 × 2 × 10 factorial experiment, with 3 batches of commercial PGM products stored at 2 temperatures (4°C and 25°C) and 10 storage periods (0, 3, 7, 14, 21, 30, 60, 90, 120 and 180 d). Three serotypes of *Salmonella* pathogens (*Salmonella* Agona, *Salmonella* Enteritidis, and *Salmonella* Tennessee) were obtained from the University of Georgia, Culture Bank, Athens, GA. A 10 g PGM sample in duplicate was inoculated as following steps: A 5 g PGM sample was placed in a sterile 120 mL amber glass bottle and 0.1 mL fresh culture *Salmonella* was inoculated to the PGM sample, then additional 5 g sample was added to the inoculated sample, and shaken vigorously to distribute the inoculum homogeneously. All inoculated and non-inoculated samples were stored at 2 temperatures and 10 storage periods, and examined the survivability of the pathogens in the PGM products. Results showed that the initial *Salmonella* counts were 7.103 log cfu/g at both temperatures. The viable *Salmonella* counts greatly decreased at the first 3 d by about 0.94 and 1.40 log cfu/g at 4°C and 25°C, respectively, and then the same levels were sustained 14 d. Further reduction occurred about 0.32 log cfu/g at 4°C and about 0.61 log cfu/g decrease at 25°C at 30 d storage. Continuous reductions were observed at 6-mo storage with additional 0.29 and 0.69 log cfu/g, respectively, at 4°C and 25°C treatments. At 180 d, the samples stored at 4°C had 1.15 log cfu/g greater survival of *Salmonella* pathogen than those stored at 25°C. It was concluded that *Salmonella* pathogens in the PGM products stored at 4°C for 180 d survived better than those stored at 25°C with the same a_w and moisture content.

Key Words: *Salmonella*, survivability, powder goat milk

W30 Effect of ultra-high-pressure homogenization and pH on buttermilk.

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Buttermilk (BM) is defined as the by-product of butter making. BM is of a particular interest due to the composition of the milk fat globule membrane (MFGM) rich in phospholipids (PL). While interesting for their nutritional and emulsifying properties, the presence of PL impairs the techno-functional properties of BM in dairy applications. Treatments to improve BM functionality are necessary to allow its valorization in dairy products. Consequently, this project aims to test the combination of ultra-high-pressure homogenization (UHPH) and pH modification on BM. Raw and pasteurized BMs, initially at pH 6.7, were produced, adjusted at pH 8.5 and 10.5, before application of UHPH at 100, 200, and 300 MPa. Changes in particle sizes were followed by laser diffraction. Protein profiles were determined by SDS-PAGE on BM and their corresponding supernatants recovered after centrifugation (70000xg/60 min) of the casein micelles, MFGM, and protein aggregates. Structural changes induced by UHPH were also monitored by TEM. For the non-homogenized BM, laser diffraction measurements showed an increase in particle mean diameter while increasing pH from pH 6.7 to 10.5, for raw BM it increased from 0.324 ± 0.044 µm to 0.700 ± 0.084 µm, and from 0.198 ± 0.017 µm to 0.302 ± 0.049 µm for pasteurized BM suggesting more severe changes in casein micelle structure and protein aggregation in raw BM. In fact, the supernatant protein profiles of the non-homogenized BM showed an increase in casein band intensity as the pH increase, indicating casein micelle dissociation, while no casein micelle dissociation was observed for the control BM. Moreover, for the BM treated at 300 MPa, the amount of soluble caseins recovered in the supernatant was markedly higher compared with the non-homogenized BM. These latest changes were more intense in pasteurized BM than raw BM. This result demonstrated that a 300 MPa UHPH treatment at pH 10.5 induced a drastic disruption of the casein micelles as confirmed by TEM. This study indicated that combination of UHPH and pH can be used to modify BM constituents, especially the casein micelles and open the door for new applications for the valorization of BM in dairy products.

Key Words: ultra-high-pressure homogenization, milk fat globule membrane, casein micelle

W31 Effect of heat treatments in the degradation of antibiotics in milk.

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Antibiotics are used in dairy farms for the treatment and prevention of diseases in dairy cattle, such as mastitis, lameness, respiratory infections and diarrhea. Antibiotic residues in milk can generate problems in the population in the form of allergies and antimicrobial resistance, as well as in industry and the environment. There are international regulations that prohibit the processing of milk with antibiotics. The relevance of possible heat-inactivation of β-lactam and tetracyclines residues in milk for the final disposal or food safety is important when making decisions when the milk is contaminated. The aim of this work was to study the effect of temperature and time on the degradation of 15 β-lactam antibiotics and 3 tetracyclines in milk using an HPLC-MS/MS. The trials were performed on raw whole milk contaminated with 100 ppb antibiotic concentrations. The effect of different temperatures (63 and 80°C) was studied during 2 different times (30 and 60 min). The degradation percentages of β-lactam and tetracyclines in milk are shown in Table 1. The results indicate that milk subjected to a thermal treatment of 63°C for 30 min, 11 of the 18 antibiotics studied degrade less than 25%, higher percentages were found in tetracycline (43%), cefoperazone (44%), cephapirin (49%), cefuroxime (53%) and cefacetrile (62%). Degradation percentages greater than 95% were found with the use of 80°C for 60 min, in cephapirin, cefacetrile, cefuroxime and cefoperazone. The knowledge of the effects of different thermal treatment conditions on antibiotic residues in milk will allow the development of tools and possible strategies that minimize risks to the environment and food safety.

Table 1 (Abstr. W31).

	Degradation[1] (%)			
	63°C		80°C	
Antibiotic	30 min	60 min	30 min	60 min
Amoxicillin	15^{a}	15^{a}	18^{a}	38^{a}
Dicloxacillin	27^{a}	22^{a}	24^{a}	47^{b}
Cloxacillin	18^{a}	14^{a}	14^{a}	34^{b}
Ampicillin	13^{a}	14^{a}	19^{a}	39^{b}
Penicillin G	20^{a}	17^{a}	22^{a}	27^{a}
Oxacillin	12^{a}	10^{a}	10^{a}	31^{a}
Cefapirin	49^{a}	50^{a}	85^{b}	97^{c}
Cephalexin	15^{a}	26^{b}	44^{c}	67^{d}
Cefazolin	13^{a}	11^{a}	10^{a}	32^{b}
Cefalonium	24^{a}	20^{a}	49^{b}	73^{c}
Cefacetrile	62^{a}	71^{a}	87^{b}	100^{b}
Cefuroxime	53^{a}	54^{a}	81^{b}	95^{b}
Ceftiofur	29^{a}	30^{a}	52^{b}	70^{c}
Cefoperazone	44^{a}	48^{a}	80^{b}	96^{b}
Cefquinome	24^{a}	26^{a}	37^{b}	64^{c}
Oxytetracycline	23^{a}	30^{ab}	37^{b}	67^{c}
Tetracycline	43^{a}	44^{a}	45^{a}	66^{b}
Doxycycline	24^{a}	26^{ab}	29^{b}	51^{c}

${}^{a\text{–}d}$Different superscripts in the same row indicate significant differences ($P < 0.05$).

[1] Values are means of triplicate analysis.

W32 Elucidation of the effect of static and stirring heating conditions on the fibrillation of native whey proteins. G. Rathod* and J. Amamcharla, *Kansas State University*, *Manhattan, KS*.

Whey proteins (WP) got considerable attention due to their compatibility for structural modification. Conversion of globular WP to fibrils can impart novel functional properties. Several researchers have prepared fibrils by heating (75–120°C) acidified WP solutions up to 20 h in static condition and reported that 80°C for 20h as an optimum condition for fibrillation of WP. However, longer heating times can limit the practical utility and commercial scale-up. The study was aimed to compare the native WP (nWP) fibrils produced under static heating (STH) and stirring heating (SRH) conditions in terms of apparent viscosity (AP), Thioflavin T fluorescence (ThT), particle size, and fibril morphology. The protein solution (2% wt/wt) was prepared by mixing nWP in distilled water, adjusted the pH 2, and heated in a water bath at 80 ± 0.5°C for up to 20 h. For STH, the nWP solution was kept undisturbed during heating while SRH was stirred using magnetic stirring at 40 rpm during heating. A representative sample was collected every hour from 2 h to 20 h and cooled immediately. The experiment was conducted in duplicate. The ThT analysis showed a steady increase in both the heating methods. At 10 h, STH produced 278 ± 39 AU while SRH produced 418 ± 41 AU indicating a higher degree of fibrillation with SRH. All the fibril-containing samples showed a shear thinning behavior. The AP also increased as the fibrillation proceeded during heating under both the conditions. The AP at a shear rate 120 s^{-1} was found to be 2.8 ± 0.7 and 4.4 ± 0.1 mPaS at 10h for STH and SRH, respectively. The heating type did not significantly ($P < 0.05$) influence the particle size during fibrillation process. It can be confirmed the growth of nWP fibrils during heating from the transmission electron micrographs. Higher degree of aggregation of fibrils was observed for STH compared with SRH method. In conclusion, heating under constant stirring potentially reduce the time required to produce matured nWP fibrils.

Key Words: native whey protein fibrils, thioflavin T, viscosity

W33 Sensory evaluation of gabiroba (*Campomanesia xanthocarpa*) whey-buttermilk fermented beverages. L. Damasceno*[1], R. T. Pfrimer[1], K. Merz[1], C. F. Cardoso[2], J. C. R. S. More[1], E. Arnhold[3], C. S. Prado[1], E. S. Nicolau[1], and C. Gebara[1], [1]*Food Research Center, School of Veterinary Medicine and Animal Science, Federal University of Goiás*, *Goiânia, Goías, Brazil*, [2]*School of Agronomy, Federal University of Goiás*, *Goiânia, Goías, Brazil*, [3]*School of Veterinary Medicine and Animal Science, Federal University of Goiás*, *Goiânia, Goías, Brazil*.

Fermented dairy foods are widely consumed around the world and in this food category new functional products have been developed. Sustainable food production is encouraged by United Nation by 2030 Agenda, and the use of whey and buttermilk in fermented beverages results in a functional and sustainable product. The aim of this work was evaluating sensory acceptance of gabiroba (*Campomanesia xanthocarpa*) whey-buttermilk fermented beverages. Thirteen formulations were produced with a variation of the ingredient's concentration: 40 to 56% of milk, 0 to 44% of whey and buttermilk and 10 to 20% of gabiroba pulp and all beverages were added of 20% of sugar. Sensory profiles of appearance, color, aroma, texture, flavor and the overall impression were evaluated by 120 untrained assessors by the 9-point structured hedonic score, where 1 = "dislike extremely" and 9 = "like extremely." The purchase intention was measured on a 5-point score, where 1 = "definitely would not buy" and 5 = "definitely would buy." The results were evaluated by Kruskal-Wallis and adjust. fdr mean comparison test ($P < 0.05$). Results showed a significant difference ($P < 0.05$) between formulations, where the lower concentration of gabiroba pulp obtained higher notes for appearance, color, and flavor. Although the gabiroba pulp has antioxidant properties the intense natural yellow color of the pulp is perceived by consumers as an artificial characteristic, resulting in lower acceptance. The residual bitterness and astringent flavor was noted on formulations with higher pulp concentration, decreasing acceptance. Higher milk concentration improved the consumer's perception of flavor and appearance. All formulations were well accepted by consumers with scores between 6 ("like slightly") and 8 ("like much") for overall impression, and for purchase intention, scores were about 4 ("I probably would buy"). Results showed that the use of whey and buttermilk, which are dairy coproducts and environmental pollutants, is suitable for dairy fermented beverages and results in a sustainable, functional, innovative product and it has become attractive for consumers.

Key Words: dairy coproduct, innovative and functional food, exotic fruit

W34 Developing probiotic butter by incorporating encapsulated probiotic organisms. S. Minj[1,2] and S. Anand*[1,2], [1]*Midwest Dairy Foods Research Center*, *Brookings, SD*, [2]*South Dakota State University*, *Brookings, SD*.

Technological advancements have enabled the addition of probiotics to many types of foods. However, maintaining the viability of probiotic organisms is generally recognized as a challenge. In our previous studies, a spray-dried formulation was prepared by microencapsulating a combination of probiotic organisms (1:1), *Bifidobacterium animalis* ssp. *lactis* ATCC27536 and *Lactobacillus acidophilus* ATCC4356, in a conjugated whey protein hydrolysate (WPH10-maltodextrin) matrix. In the current study, a "probiotic butter" was developed by incorporating the conjugated-WPH encapsulated probiotics. Butter was prepared using heavy whipping cream (36% fat) in a KitchenAid mixer, under lab conditions. The cream was churned until the butter grains separated from the buttermilk, which was completely drained off to collect the grains. The butter grains were inoculated with the spray-dried formulation (probiotic population; 8.98 ± 0.02 log cfu/g) at 0.1 and 1% levels and worked to a homogeneous product. For salted butter, the mixture was added with 1.5% salt before working. The survival of the probiotic bacteria in the probiotic butter was determined using MRS agar with added L-cysteine (0.05%), incubated at 37°C in anaerobic jars with gas packs. Three trials were conducted, and the samples were drawn in triplicates. One-way ANOVA was applied to differentiate the mean values. The study revealed comparable probiotic counts in both salted and unsalted butter ($P > 0.05$). The mean counts observed in unsalted probiotic butter were 5.13 ± 0.05 and 8.22 ± 0.02 log cfu/g at 0.1% and 1%, respectively, whereas in salted probiotic butter, the mean counts were 5.09 ± 0.04 and 8.13 ± 0.01 log cfu/g at 0.1% and 1%, respectively. Hence, the study provides a proof of concept for the potential to develop a probiotic butter with desired number of viable probiotic organisms. Further studies are in progress to determine the survival of encapsulated probiotics in probiotic butter samples during refrigerated storage.

Key Words: whey protein, encapsulation, probiotics

W35 Types of *Bacillus* endospores in milk protein concentrate and milk protein isolate. S. Jha[1,2], N. Singh*[1,2], and S. Anand[1,2], [1]*Midwest Dairy Foods Research Center*, *Brookings, SD*, [2]*South Dakota State University*, *Brookings, SD*.

Dairy powders and ingredients have an enormous market worldwide. Several studies indicate the presence of aerobic spore formers and bacterial endospores in dried milk powders. The present study investigates the types of *Bacillus* endospores present in milk protein concentrates (MPC) and milk protein isolates (MPI). Twenty-five samples each of MPC and MPI were obtained from commercial dairy plants and were enumerated for 3 types of endospores after heat treating at 80°C/12min for regular spores (SP), 100°C/30min for high-heat resistant spores (HHRS), and 106°C/30min for specially thermoresistant spores (STS). In all these cases, 11 g of the sample was reconstituted in 99 mL of phosphate buffer saline, and the desired serial dilutions were plated on tryptic soy agar. The plates were incubated under aerobic conditions for 24 h at 37°C for mesophiles, and 55°C for thermophiles. Colonies were differentiated based on colony morphologies and the distinct isolates were identified using MALDI-TOF. All samples were tested in duplicates and means were compared using ANOVA. In MPC, the samples containing mesophilic spores were 39% SP, 23% HHRS, and 38% STS. Similarly, the samples containing thermophilic spores were 43% SP, 22% HHRS, and 35% STS. In MPI samples, the mesophilic spore pattern showed 39% SP, 29% HHRS, and 32% STS, while the samples with thermophilic spores were 54% SP, 31% HHRS, and 15% STS. Based on the percent distribution, SP was the most prominent heat treatment spore-category. The MALDI-TOF identification revealed *Bacillus licheniformis* to be the most predominant spore former species in both MPC (51%) and MPI *(41%)* samples. These findings will help design process interventions for controlling endospores in powders, and decide their utilization based on the types of spore-types encountered.

Key Words: endospore, milk protein concentrates, milk protein isolates

W36 Psychrotolerant spore-forming bacterial spoilage of HTST milk pasteurized for 20 seconds at 75, 85, or 90°C and stored at different temperatures. T. Lott*, N. Martin, S. Murphy, A. Trmcic, and M. Wiedmann, *Cornell University*, *Ithaca, NY*.

In the absence of post-pasteurization contamination (PPC), psychrotolerant, aerobic, gram-positive spore-forming bacteria (PASB) is what typically limits the shelf-life of fluid milk. Reducing spoilage due to PASB is difficult as these bacteria enter the fluid milk continuum in the raw milk and can survive HTST pasteurization. Currently, several strategies are used to reduce the spore levels in raw milk, which may include bactofugation, microfiltration, and controlling the entry of the spores into raw milk on the farm. The goal of the project was to see if shelf-life extension could be achieved with higher HTST temperatures. Approximately 70 L of raw milk from Texas were processed on a pilot-plant scale at 75°C/20s, 85°C/20s, or 90°C/20s. Pasteurized milk from each HTST temperature was then stored at 3°C, 6.5°C, or 10°C, and microbiological quality was monitored through 42 d or until samples reached maximum bacterial concentration. HTST milk was tested for (1) total bacteria count, (2) gram-negative count to test for the absence of post-pasteurization contamination, (3) pH, and (4) particle analysis for the confirmation of coagulation. Additionally, a sample of raw milk was taken to measure total bacteria count, mesophilic spore count (MSC), psychrotolerant spore count (PSC), and total bacteria count after preliminary incubation (13°C for 18h). Results of a one-way ANOVA showed there was no significant difference ($P > 0.05$) between total bacterial counts by HTST temperatures within each storage temperature over shelf-life. These results suggest that since there is no extension of shelf-life using HTST pasteurization temperatures above 80°C, 75°C is the optimal HTST pasteurization temperature tested as it requires the least energy input and based on other studies would result in reduced risk of cooked sensory defects. Further, it was found that bacterial concentrations exceeded 1,000,000 cfu/mL (where consumers typically begin to sense defects) before changes in other indicators of sensory characteristics were observed (i.e., pH and coagulation).

Key Words: spore, fluid milk, psychrotolerant

W37 Selective survival of dairy cultures to high-pressure processing by leveraging freeze-drying and encapsulation. M. McGillin* and S. Alcaine, *Cornell University*, *Ithaca, NY*.

High-pressure processing (HPP) is an increasingly adopted non-thermal treatment for food products. Although effective at inactivating vegetative cells, spores can survive and potentially germinate, hence the need for additional hurdles to address them. Biopreservation utilizes select food-grade microbes to inhibit the growth of undesired microbes. Furthermore, in many low acid foods, such as dairy, a subsequent selective culture activity is desired. Currently, adding desirable microbes to a food matrix before HPP inactivates them. A potential way to circumvent this issue is to maintain the cultures in a desiccated, spore-like state, which enables them to resist the damaging effects of HPP. For application in a high moisture food, an encapsulation system that maintains desiccation prior and during HPP, followed by rehydration post-HPP is required. To evaluate the survival of desiccated cultures to HPP treatment, cell counts of freeze-dried lactic acid bacteria (LAB) cultures (HPP-FD-LC) and hydrated LAB cultures (HPP-LC) were compared. There was approximately a 7.5 log difference between HPP-FD-LC (6.27×10^8 cfu/mL) and HPP-LC (3.29 cfu/mL) post-HPP (600 MPa, 3 min, 5°C), demonstrating the role desiccation plays in HPP survival. To delay the hydration of the HPP-FD-LC in a high-moisture food matrix, the cultures were encapsulated in cocoa butter (33°C) by homogenization before HPP, resulting in cocoa butter encapsulated freeze-dried cultures (CB-FD-PC). A response surface model was generated to optimize homogenization factors of temperature, speed, and duration. Analysis of the model determined the optimal factors to be 4000 rpm for 3 min at 33°C. The only significant factor ($\alpha < 0.05$) proved to be the starting temperature, demonstrating a relationship between lower temperatures and higher viability. Future work will determine the rate of rehydration of the encapsulated cultures within a high-moisture food matrix (i.e., skim milk), to establish the processing window in between introducing the encapsulated cultures into the food matrix and the HPP treatment.

Key Words: high-pressure processing, protective cultures, encapsulation

W38 The effect of following organic or conventional dairy farming practices on the raw milk microbiome. D. Van De Grift*, G. Angima, L. Goddik, J. Cruickshank, and S. Park, *Oregon State University*, *Corvallis, OR*.

Fluid milk consumption has seen a decrease in consumer demand over the last 10 years, while dairy commodities (i.e., cheese) has been increasing. Along with consumers' demands, organically produced dairy products are gaining in popularity than conventionally produced products. These consumer trends demand that a high standard of raw milk quality be met by farmers. The aim of this study was to assess how following organic or conventional farming practices affect the microbiome of raw milk. Four organic and 4 conventional farms having between 100 to 260 and 300 to 450 milking cows respectively were selected from, and located within Oregon's Linn and Marion counties. Bulk tank (BT) milk samples were collected along with environmental samples, 3 different visit per quarter (sampling period). Each sampling period is the first month of each quarter (January, April, July, and October). Sampling the environment of the farms at the same time as the BT sample, furthers the understanding of how environmental microorganisms can influence the raw milk supply. The environmental samples were collected from feed, current bedding, fresh bedding, and a swab of a towel used in the milking parlor. Aerobic and coliform plates were performed immediately on milk samples upon arrival to the lab, and 16S rRNA sequencing based on V4 region in raw milk and environmental samples was performed once all samples were collected for each quarter. The 1st quarter data suggest that when both organic and conventional herds are contained in a barn that there is more coliform and anerobic bacteria in conventional milk than in organic BT samples ($P < 0.05$). There is no difference between BT bacterial counts and bedding material type from either organic or conventional farms ($P > 0.05$). Using 16S rRNA sequencing in addition to the bacterial plating has provided a detailed description of the bacterial differences on each farming site, within farming groups, and between farming types.

Key Words: milk, microbiome, high-throughput sequencing

W39 Microbial counts of camel milk probiotic drinkable yogurt as affected by monk fruit sweetener. A. Buchilina*[1] and K. Aryana[2], [1]*Louisiana State University*, *Baton Rouge, LA*, [2]*Louisiana State University Agricultural Center*, *Baton Rouge, LA*.

Different sweeteners in different food matrices have different influences on microbial growth. Influence of monk fruit sweetener on yogurt culture bacteria and *Lactobacillus acidophilus* in camel milk drinkable yogurt is not clearly understood. Camel milk drinkable probiotic yogurts were manufactured with increasing levels of monk fruit sweetener (0, 3.2, 9.6, and 19.2g per 7.57 L of camel milk). Yogurts were stored at 4°C until needed and 3 replications were conducted. Counts of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* were enumerated on d 1, 7, 21, 28, 35 and 42 after manufacture. Counts of yeast and mold and coliforms were < 10 cfu/mL in all yogurt samples throughout storage. Increase in amount of monk fruit sweetener did not significantly influence counts of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* but increase in storage days significantly influenced counts of each of these microorganisms in probiotic camel milk yogurts. Counts of *Streptococcus thermophilus* peaked at d 28, and then did not significantly change during the remaining storage period. The counts of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* significantly decreased over storage period. The significant decrease in the counts of *Lactobacillus* species may be caused by the presence of antimicrobial compounds in camel milk such as lactoferrin, IgG, and lysozyme. Monk fruit sweetener did not influence the growth of starter and probiotic cultures in camel milk drinkable yogurt.

Key Words: probiotic, health, microbial

W40 Industry survey: The diversity of rework practices in fluid milk and dairy powder production. C. Rush*, J. Waite-Cusic, and L. Meunier-Goddik, *Oregon State University*, *Corvallis, OR*.

Reworking dairy products is common practice in the industry as a way to help minimize waste while reducing cost for processors. However, there are no established guidelines for reworking fluid milk and milk powders and processors have developed their own unique procedures. The objective of this survey was to investigate current industry practices for reworking dairy products. Six national dairy processors (n = 4 fluid milk; n = 2 dairy powder) were surveyed in-person or via phone interview to understand their motivation for rework, common reworked products, storage and processing parameters, and potential defects associated with reworked products. Results of the survey defined rework (e.g., reclaim, rerun, recovery) as the commingling of either a freshly processed or an unpasteurized product with another product that has been previously processed or pasteurized, and then re-pasteurized or re-processed together with a reset of the code date. For fluid milk processors, the motivation for rework may be due to date-challenged product, mislabeled containers, leaky cartons, microbial contamination, and the recovery of milk after flushing the lines from cleaning in place (CIP) or when standardizing the fat content between products. Dilution rates for most products were < 20% with more stringent limits (<10%) on more spoilage prone products (e.g., chocolate milk). Processors reported quality issues of reworked product, including premature spoilage, flavor defects, and high plate counts. Dairy powder processors often rework due to microbial contamination or a deviated specification (e.g., low/high pH, moisture content) at 3–10% depending on rework motivation. Dairy powders may be reconstituted with fresh milk or water and re-dried, while others are reworked after the drying stage. Reworked dairy powders often show increased spore counts and scorch particles, causing some products to be downgraded or barred from exportation. The data collected from this survey will be used to guide further investigations on the quality impacts of reworking fluid milk and milk powders.

Key Words: rework, milk, powder

Forages and Pastures 2

W41 Yield and quality of conventional and brown midrib (BMR) pearl millet with different establishment dates or harvested at different maturities. M. Oskey[1], B. Stancil[2], and M. J. Aguerre*[1], [1]*Department of Animal and Veterinary Science, Clemson University, Clemson, SC*, [2]*Variety Testing and SC Crop Improvement, Clemson University, Clemson, SC.*

The main objectives of these studies were to evaluate the yield, composition and digestibility of conventional and brown midrib (BMR) pearl millet (PM) with different establishment dates or harvested at different maturity stages. A second objective was to evaluate the impact of mixing PM with cowpea on measured variables. Two trials were conducted in field plots (1.5 m × 6.1 m) as randomized complete block designs with a split plot arrangement of treatments. In trial 1, 2 varieties of PM (conventional and BMR) were planted at 2 different dates (14d apart) and harvested at early heading stage. In trial 2, 2 varieties of PM (conventional and BMR), mixed or not with cowpea were harvested at PM boot or heading stages. Samples from both trials were analyzed for CP, NDF, sugars (as % DM) and 30-h in vitro NDF digestibility (IVNDFD as % NDF). In trial 1, dry matter yield was numerically lower for BMR compare with conventional PM (4,255 vs. 3,875 kg/ha, $P = 0.16$), but delaying establishment significantly ($P < 0.01$) reduced DM yield by 23%, regardless of PM variety. Nutrient composition was similar between PM varieties (15.8% CP, 52.0% NDF, and 7.0% sugars). However, BMR had higher IVNDFD (66.3 vs. 63.6%) compared with the conventional variety. In Trial 2, DM yield was 8.3% lower for BMR compare with conventional PM (3,266 vs. 3,875 kg/ha, $P = 0.07$). Similarly, mixing PM with cowpea tended to also reduce DM yield by 8%. Harvesting at heading stage numerically increased DM yield by 5.3%. Pearl millet with the BMR trait had a higher IVNDFD, regardless of mixing it or not with cowpea (64.6 vs. 60.0%; $P < 0.05$). More mature PM resulted in a 7.3% lower IVNDFD (59.9 vs. 64.8%; $P = 0.04$). Nutrient composition was similar between forages (51.9% NDF, and 8.5% sugars), but cowpea increased CP level (14.2 vs. 15.3%, $P = 0.01$) when mixed with both PM varieties. Results of this studies suggest that BMR PM is more digestible than conventional but at expenses of lower DM yield. Planting date and plant maturity are important tools to manipulate PM yield and quality but mixing cowpea with PM had small effects on quality at expenses of yield.

Key Words: digestibility, summer annual, forage

W42 Evaluation of individual amino acids (AA) and factors that affect the AA profile in corn silage over a 2-year period. X. Huang*[1], P. Yoder[2], and R. Ward[1], [1]*Cumberland Valley Analytical Services, Waynesboro, PA*, [2]*Perdue AgriBusiness LLC, Salisbury, MD.*

Balancing diets for essential AAs has both environmental and economic benefits for the dairy industry, and this approach requires accurate AA profiles of feedstuffs. The objectives of this study were to assess NIR measurements for individual AAs and factors that may affect the EAA profile of corn silage. The fermentation results and nutrient components data were collected from 1,243 corn silage samples across 2 years (2018 and 2019). Around 13% outliers were removed using the 3-step method published by Yoder et al. (2014). Analysis methods (NIR vs chemistry fermentation) had no effect on nutrient composition ($P > 0.05$). The sampling year had a significant effect on nutrient composition, with higher CP, starch, ammonia and dry matter concentrations but lower NDF for 2019 corn silage compared with 2018 ($P < 0.05$). Averaged AA content in corn silage was 1.65 ± 0.11, 4.75 ± 0.23, 3.14 ± 0.12, 8.59 ± 0.55, 4.76 ± 0.34, 1.56 ± 0.22, 2.59 ± 0.31, 1.75 ± 0.44, 3.37 ± 0.21% of CP for Met, Val, Ile, Leu, Phe, His, Lys, Arg and Thr respectively, which was similar to CNCPS and NRC 2001 library values. All EAAs except for Met, Val, Arg and Thr were affected by sampling year. The Leu, Ile, Phe and His as a % of CP were higher in 2019 corn silage, while Lys as % of CP was higher in 2018 corn silage, which was likely associated with ammonia and starch contents. For example, Leu was positively correlated (0.74) with starch while Lys and Arg were negatively associated with ammonia (−0.71 correlation for both AA). The EAA profile likely is related to corn kernel content versus plant content in corn silage considering the different EAA profile present in these 2 parts of corn silage (12.0 vs. 8.5 Leu as % of CP for corn grain vs. corn silage (CNCPS, v6.5)). Regression models were derived and for some EAA, nutrient components could be used to predict the variation observed in EAA as a percent of CP. In summary, the amount of starch (i.e., corn grain vs. plant material) and ammonia concentration affected individual EAA profile of corn silage protein. Consideration for this variation present maybe warranted for improving our ability to balance diets for EAA.

Key Words: AA profile, corn silage, NIR

W43 Milk production and composition in Jersey cows grazing forage canola. L. H. P. Silva*[1], J. P. Sacramento[2,1], D. C. R. Gomez[1], Y. Geng[3], M. Ghelichkhan[1], S. L. Dillard[4], K. J. Soder[5], and A. F. Brito[1], [1]*University of New Hampshire, Durham, NH*, [2]*Universidade Federal de São João del Rei, São João del Rei, MG, Brazil*, [3]*Chinese Academy of Agricultural Sciences, Beijing, China*, [4]*Auburn University, Auburn, AL*, [5]*USDA-Agricultural Research Service, Pasture Systems and Watershed Management Research Unit, University Park, PA.*

Canola herbage is a high-quality annual forage that can be used to extend the fall grazing season due to its winter hardness. However, there is scarce information evaluating canola as a grazing forage for dairy cows. We aimed to evaluate the effect of partially replacing baleage with canola herbage on milk production and composition. Twelve multiparous and 8 primiparous mid-lactation Jersey cows were blocked by parity and, within block, assigned to control (CTRL) or canola (CAN) diet in a randomized complete block design. Cows in the CTRL group were kept in confinement, while CAN cows stayed in the barn during the day and had access to pasture from 1800 to 0500 h. Diets were formulated to yield (DM basis) 60:40 forage:concentrate ratio with 67% of the baleage replaced by canola herbage in the CAN diet. The experiment was conducted during fall 2019 and lasted 7 wk (2-wk covariate) with sample collection done during wk-3 and wk-5. Data were analyzed with repeated measures in SAS. The canola paddock was divided into strips to provide daily herbage allowance of 19 kg of DM/cow. Canola herbage yield 6,662 kg of DM/ha, with 24.1% CP, 42.2% NFC, and 16.5% aNDFom. As expected, CAN cows consumed 37% less TMR than CTRL (12.4 vs. 19.6 kg/d of DM). Estimated herbage DMI (pre- minus post-grazing mass) averaged 7.62 kg/d. Milk yield, FCM, and ECM did not differ between diets. Milk true protein concentration was greater in CAN than CTRL, but milk protein yield was similar between diets. While milk fat content was not affected by diets, milk fat yield tended to decrease with feeding CAN (1.08 vs. 1.01 kg/d; $P = 0.08$), which may be associated with the low aNDFom in canola herbage compared with baleage. Feeding CTRL elevated milk lactose concentration relative to CAN (4.70 vs. 4.61; $P < 0.01$) without change in milk lactose yield. A diet by week interaction was found for MUN, with CTRL cows showing greater MUN in wk-3 than wk-5 (15.1 vs. 12.2 mg/dL) but no change was observed for CAN. Milk SCS was greater in CAN than CTRL ($P = 0.05$). Overall, canola herbage can partially replace up to 67% of baleage in the diet DM without negative effects on milk yield and composition.

Key Words: brassica, grazing, milk production

W44 Effect of different measurement methods on predicted dry matter content in whole-plant corn forage and silage. E. C. Diepersloot*[1], E. M. de Paula[1,2], M. R. Pupo[1], L. G. Ghizzi[1,3], C. Heinzen Jr.[1], C. L. McCary[1], and L. F. Ferraretto[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL*, [2]*Instiuto de Zootecnia, Centro APTA Bovinos de Corte, Sertãozinho, São*

Paulo, Brazil, [3]*Department of Animal Nutrition and Animal Production, University of São Paulo*, *Pirassununga, São Paulo, Brazil.*

This study aimed to evaluate the effectiveness of portable Near-Infrared Spectrometers (pNIRS) and Koster ovens in predicting DM content of whole-plant corn forage (WPCF) and silage (WPCS). Experiments 1 and 2 compared 3 methods of measuring DM of WPCF and WPCS, respectively, as follows: 1) a forced-air oven set at 60°C for 48 h (gold standard), 2) Koster ovens weighed after 30 min then every 10 min until reaching a constant weight (change of less than 0.1g), and 3) pNIRS (average of 10 scans). Experiment 3 was an on farm trial and measured DM content of WPCF samples with Koster ovens and pNIRS. Following farm protocol, Koster ovens were weighed after 40 min then every 10 min until reaching a constant weight (change of less than 0.5g). Data were analyzed using Proc Glimmix and Proc Corr of SAS. In experiment 1, the DM average for Koster ovens (34.3%) and forced-air oven (35.5%) did not differ ($P = 0.39$); but the DM average for all 3 pNIRS (30.6, 31.0, and 30.5%) were lower ($P \leq 0.05$) than other methods. Additionally, all other treatments were correlated with the forced-air oven ($R^2 \geq 0.85$). In experiment 2, the DM of the forced-air oven (34.1%) and Koster oven (33.3%) did not differ ($P \geq 0.08$) from pNIRS 2 (33.3% DM). The pNIRS 3 treatment (33.2% DM) only differed from the forced-air oven ($P = 0.05$). Forced-air oven and Koster oven were correlated ($R^2 = 0.82$), while the pNIRS were not correlated with either ($R^2 \leq 0.30$). For experiment 3, the average DM for Koster ovens (37.3%) was greater than ($P < 0.001$) pNIRS 1 (32.5%), pNIRS 2 (33.7%), and pNIRS 3 (33.6%) with no difference among pNIRS ($P \geq 0.19$). All pNIRS were correlated with the Koster oven ($R^2 \geq 0.80$). Under the conditions of the present study Koster ovens accurately assess DM content of WPCF and WPCS. However, pNIRS should be used with caution in WPCF as predicted DM was lower than the actual DM content. Although WPCS DM values from the pNIRS did not differ from other treatments, caution should still be used as there were differences among units and pNIRS were not correlated with other treatments.

Key Words: NIR, Koster oven, dry matter

W45 Yield and nutritive value of sorghum silage cut at different harvesting heights. J. Granados-Niño[1], J. Sánchez-Duarte[2], D. Reta-Sánchez[2], O. I. Santana[2,3], M. López-Calderón[1], E. Ochoa-Martínez[2], and F. Díaz*[4], [1]*FAZ-UJED*, *Venecia, Gómez Palacio, Durango, México*, [2]*INIFAP*, *Matamoros, Coahuila, México*, [3]*INIFAP*, *Aguascalientes, México*, [4]*Dairy Research Center, dellait*, *Brookings, SD*.

The aim of this study was to assess yield and nutritional value of sorghum (*Sorghum bicolor* L.) silage when harvested at 6 cutting-heights. The crop was planted in a warm and arid environment in northern Mexico, using a conventional seeding rate of 125,000 seeds/Ha, and fertilized and irrigated conforming to recommendations for the area. Plants were cut at milk-dough stage at 10, 20, 30, 40, 50, and 60 cm stubble-height, chopped to 2 mm, and packed in 1-L glass mini-silos by triplicate at a density of 261 kg DM/m^3. Data were analyzed as a completely randomized design using Fisher's protected LSD test for mean separation. Results (Table 1) showed forage DM yield was only significantly reduced at 60 cm stubble-height. Silage pH indicated cutting at 30 cm may allow for better fermentation than the other treatments. Silage DM and CP contents were not affected by cutting height, however NDF and lignin were greater when plants were cut at 10 cm stubble-height. Lignin notably declined at stubble-height greater than 20 cm. Silage estimated NE_L was only lower when cutting at 10 cm stubble-height likely associated with lignified fiber. In contrast, NDF digestibility increased at higher stubble-heights, while NDF and NEL yields were similar among treatments. Overall, these findings indicate that harvesting sorghum for silage in a range of 20–40 cm stubble-height may optimize DM yield and nutritional value.

Key Words: *Sorghum bicolor* (L.), silage, nutrients

Table 1 (Abstr. W45).

	Stubble-height at harvest (cm)						
Item	10	20	30	40	50	60	LSD
DM yield (t/ha)	18.69^{a}	18.50^{a}	18.12^{a}	17.76^{a}	17.27^{ab}	16.12^{b}	1.55
Silage pH	4.39^{ab}	4.89^{a}	3.96^{b}	4.71^{ab}	4.68^{ab}	4.12^{ab}	0.86
DM (% of silage)	29.42	29.46	29.27	29.50	29.66	29.57	0.58
CP (%)	6.37	6.50	6.07	7.23	6.27	6.37	1.37
NDF (%)	74.13^{a}	66.85^{b}	66.15^{b}	64.00^{b}	63.03^{b}	62.87^{b}	5.60
Lignin (%)	8.12^{a}	7.70^{a}	6.35^{b}	6.85^{b}	6.42^{b}	6.46^{b}	0.83
$NDFD_{30h}$ (% NDF)	27.43^{d}	29.33^{cd}	30.23^{c}	34.19^{b}	35.59^{b}	38.64^{a}	2.11
$NDFD_{30h}$ yield (t/ha)	3.80	3.62	3.63	3.90	3.88	3.92	0.65
NE_L (Mcal/kg DM)	1.0^{b}	1.09^{ab}	1.09^{ab}	1.16^{a}	1.18^{a}	1.16^{a}	0.12
NE_L yield (Mcal/ha)	18,659	20,094	19,808	20,548	20,371	18,684	2,448

[a–d]Mean difference using Fisher's protected LSD test ($P < 0.05$).

W46 A survey of wildfire ash impacts on California's forage crops. B. Karle*[1], L. Forero[2], J. Davy[3], J. Harper[4], D. Macon[5], J. Heguy[6], N. Clark[7], J. Stackhouse[8], T. Schohr[9], D. Lile[9], E. DePeters[10], D. Meyer[10], and R. Poppenga[11], [1]*University of California Cooperative Extension*, *Orland, CA*, [2]*University of California Cooperative Extension*, *Redding, CA*, [3]*University of California Cooperative Extension*, *Red Bluff, CA*, [4]*University of California Cooperative Extension*, *Ukiah, CA*, [5]*University of California Cooperative Extension*, *Auburn, CA*, [6]*University of California Cooperative Extension*, *Modesto, CA*, [7]*University of California Cooperative Extension*, *Hanford, CA*, [8]*University of California Cooperative Extension*, *Eureka, CA*, [9]*University of California Cooperative Extension*, *Quincy, CA*, [10]*University of California Department of Animal Science*, *Davis, CA*, [11]*University of California Animal Health and Food Safety Laboratory*, *Davis, CA*.

The potential impacts of wildfire ash deposition on crops harvested for livestock feed are largely unknown, especially relative to burned structures containing unknown levels of contaminants from household products, vehicles, businesses and residential chemicals. In 2018, several devastating fires plagued California, resulting in ashfall with an unknown toxicological makeup. A survey of 26 irrigated pastures, 20 hay piles, and 16 corn silage piles from locations either affected or not affected by wildfire ash was conducted. All samples were analyzed for heavy metals and a subset (n =

38) were analyzed for toxicological compounds by gas and liquid chromatography–mass spectrometry organic chemical screens. Toxicological compounds in harvested forage were detected in 6 fields (4 affected by ash, 2 not affected by ash). To determine the effects on heavy metal levels, a type 3 sums of squares multiple ANOVA was run using factors of forage source (irrigated pasture, hay, silage), impact of ash (Y/N), and their interaction. Lead, mercury, arsenic and cadmium were not detected in any samples. Models were run individually for copper, manganese, zinc, and iron. Multiple range tests used Fisher's least significant difference test. Table 1 shows the least squares means metal levels by forage type and impact of ash. Copper was not significantly affected by forage type (P = 0.07), but was higher in fields not affected by ash (P = 0.03). Zinc was affected by forage source (P < 0.01), but not by the impact of ash (P = 0.32). Manganese was significantly impacted by forage source (P = 0.04), but not by ash (P = 0.12). Iron was impacted by forage source (P = 0.02), but not by the impact of ash (P = 0.19). These results indicate that forages affected by wildfire ash deposition are safe for livestock to consume.

Table 1 (Abstr. W46). Least squares means mineral levels by forage source and impact from ash

Item	CU, ppm	Zn, ppm	Mn, ppm	Fe, ppm
Forage source				
Pasture	4.4^{a1}	12.3^{a}	62.8^{b}	94^{a}
Silage	15.1^{b}	10.1^{a}	12.9^{a}	80.9^{a}
Hay	7.7^{ab}	21.9^{b}	54.0^{b}	291^{b}
Impacted by ash				
Yes	4.9^{a}	13.6^{a}	30.6^{a}	199.0^{a}
No	13.2^{b}	15.9^{a}	55.9^{a}	111.5^{a}

^{a,b}Within a column, levels with the same letter are not different at 0.05.

Key Words: wildfire, forage quality, toxicology

W47 Relationship of undigested and physically effective neutral detergent fiber with dry matter intake and energy-corrected milk yield of Holstein cows.

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Forage quality measures should be related to animal performance. Undigested neutral detergent fiber after 240 h of in vitro fermentation (uNDF240) is a laboratory measure of indigestible NDF that is related to rumen fill and NDF turnover. Physically effective uNDF240 (peuNDF240) is the product of uNDF240 and physical effectiveness factor (% of particles ≥ 1.18-mm screen with dry sieving). Our objective was to build a database to develop relationships between uNDF240 and peuNDF240 with dry matter intake (DMI), energy-corrected milk yield (ECM), and rumen pH in Holstein cows. We used Pearson correlations and multiple linear regression (MLR) on 16 treatment means from 5 experiments conducted at the William H. Miner Agricultural Research Institute in Chazy, New York. Diets contained corn silage, hay crop silage, chopped wheat straw, and 2 diets contained substantial beet pulp. Cows in the studies averaged (mean ± SD) 104 ± 15 d in milk, 27.7 ± 1.1 kg/d DMI, and 48.7 ± 2.2 kg/d ECM yield. Data were analyzed using Reg and Corr procedures of SAS (version 9.4). The uNDF240 had a strong negative (P < 0.05) relationship with DMI and ECM (r = −0.57 and −0.76, respectively) and a strong positive (P < 0.05) relationship with mean rumen pH (r = 0.82). Physically effective uNDF240 had a strong negative (P < 0.05) relationship with DMI and ECM (r = −0.78 and −0.88, respectively), and a strong positive (P < 0.05) relationship with mean rumen pH (r = 0.88). The MLR analysis to predict DMI and ECM accounted for 32.7 and 58.3% of the variance using uNDF240. The MLR analysis to predict DMI and ECM accounted for 60.4% and 77.8% of the variance using peuNDF240. Based on these results, uNDF240 and peuNDF240 are highly related to DMI and ECM in corn silage-based diets and could be used to create useful prediction models. These relationships of uNDF240 and peuNDF240 with DMI and ECM need to be investigated for legume-based diets.

Key Words: undigested fiber, dry matter intake, energy-corrected milk

W48 Corn silage quality index: An index combining milk yield and silage nutritional and fermentation parameters.

R. M. H. Tharangani[1], Y. K. Chen[1], L. S. Zhao[1], L. Ma[1], P. J. Kononoff[2], W. P. Weiss[3], and D. P. Bu*[1,4], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Department of Animal Science, University of Nebraska-Lincoln*, *Lincoln, NE*, [3]*Department of Animal Sciences, Ohio Agricultural Research and Development Center, Ohio State University*, *Wooster, OH*, [4]*CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia*, *Beijing, China.*

Development of indexes based on milk yield of lactating dairy cows when fed corn silage-based diets in combination with silage quality parameters can provide clear guidance to assess the overall quality of the corn silage produced. This study was done to determine the minimum data set (MDS) for corn silage quality evaluation among commonly used parameters for nutritional and fermentation quality and to develop an integrated corn silage quality index (CSQI) based on standard scoring functions and weight assignment approach. Principal component analysis (PCA) and multiple regression analysis (MRA) were used to determine MDS and to assign parameter weights, whereas standard scoring functions were used to normalize silage quality parameters. A variety of corn silage samples (n = 390) representing spatial and seasonal heterogeneity, were collected from 195 intensive dairy farms in China and analyzed for 16 frequently used chemical and fermentation parameters. Concurrent with silage sampling, average daily milk yield respective to each silage was collected using 50 mid-lactating dairy cows fed corn silage-based diets (i.e., 39–48% DM corn silage in TMR) and used as the dependent variable in MRA. The silage quality parameters retained in the MDS included 30-h in vitro NDF digestibility, and concentrations (DM basis) of starch, crude protein, ether extract, ammonia and lactic acid. The CSQI was developed by summing normalized and weighted quality parameters retained in the MDS and the new index was evaluated against observed daily milk yield measurements. Accuracy and precision of predicting corn silage quality using MRA based CSQI were higher than those of the PCA-based CSQI. Thus, among the 2 multivariate approaches, the MRA was suggested for future corn silage quality evaluation, and MRA based CSQI provides an index to assess relative performance among different corn silage based on the overall quality.

Key Words: corn silage, quality index, milk yield

W49 Brown midrib (BMR) forage sorghum or BMR pearl millet silage support similar production when fed along with corn silage.

J. K. Bernard* and S. Tao, *University of Georgia*, *Tifton, GA.*

A randomized complete block trial was conducted to evaluate the feeding value of silage produced from either brown midrib (BMR) pearl millet (PMS) or BMR forage sorghum (FSS). The PMS contained (DM basis) 37.2% DM, 14.2% CP, 35.4% ADF, 64.6% NDF and 44.0% 30 h NDFd. The FSS contained 29.0% DM, 11.7% CP, 38.3% ADF, 60.5% NDF and 60.5% 30 h NDFd. Thirty-two mid-lactation Holstein cows (139 ± 21 DIM) trained to eat behind Calan gates were fed a common diet based on corn silage for 2 wk preliminary period. Cows were abruptly switched to 1 of 2 experimental diets containing 32.6% of DM as corn silage and 20.6% of DM from either PMS or FSS for the following 6 wk. DMI and milk yield were measure daily and milk samples were collected during wk 4 through 6. Data were subjected to repeated measures analysis of covariance using preliminary data as a covariate. During the trial no differences (P > 0.10) were observed in DMI (average 28.6 and 28.7 kg/d) or milk yield (average 36.5 and 37.4 kg/d) between PMS and FSS, respectively. Milk yield, milk component percentage, ECM yield and dairy efficiency (ECM/DMI) were not different (P > 0.10) among treatments during wk 4 to 6 (Table 1). Cows fed diets supplemented with FSS had higher ($P < 0.0001$) MUN concentrations compared with PMS and the differences were greater for FSS during wk 4 and 6 compared with PMS resulting in a treatment by week interaction ($P < 0.0001$). No differences ($P > 0.10$) were observed in change of BW or BCS among treatments during the trial. Results of the current trial indicate that silage produced from either BMR pearl millet or forage sorghum will support similar performance of mid-lactation dairy cows when fed along with corn silage.

Table 1 (Abstr. W49). Intake and production response of cows fed diets supplemented with BMR pearl millet silage or BMR forage sorghum silage during wk 4 to 6

Item	PMS	FSS	SE	*P*-value
DMI, kg/d	26.3	26.5	0.6	0.89
Milk, kg/d	36.1	36.9	0.7	0.44
Fat, %	3.42	3.48	0.09	0.63
Protein, %	2.78	2.78	0.03	0.95
Lactose, %	4.79	4.72	0.03	0.64
SNF. %	8.47	8.46	0.03	0.10
ECM, kg/d	35.5	36.5	1.0	0.70
Efficiency, ECM/DMI	1.35	1.38	0.04	0.77
MUN, mg/dL	14.27	16.72	0.32	<0.0001

Key Words: pearl millet silage, forage sorghum silage, milk yield

Production, Management, and the Environment 3

W50 Evaluating silage quality of perennial forages seeded with annual sudangrass or sorghum-sudangrass hybrids as companion crop. S. Thevakumaran*[1], C. Matteau[2], B. Baurhoo[1,2], P. Seguin[1], and A. Mustafa[1], [1]*McGill University*, *Saint Anne De Bellevue, QC, Canada*, [2]*Belisle Solution Nutrition Inc*, *Saint-Mathias-sur-Richelieu, QC, Canada*.

The objective of this study was to evaluate the effects of a mixture of perennial forages (alfalfa, clover and tall fescue) seeded alone (control) or with different annual companion forages [sudangrass (SG), sudangrass brown midrib (BMR) gene 12 (BSG) or sorghum-sudangrass BMR gene 6 (BSSG)] on ensiling characteristics, and chemical composition and in vitro total-tract NDF digestibility (IVTTNDFD) of experimental silages. Experimental plots (8 replicates per treatments) were harvested at 60 d (1st cut) and 90 d (2nd cut) at the bud stage of alfalfa. Harvested forages at 90 d were ensiled in laboratory silos for 42 d. Forage indigestible NDF (iNDF) was calculated by in vitro incubation at 240 h whereas potentially degradable NDF (pdNDF) was calculated by subtracting iNDF from total NDF. Digestion rate (kd) of pdNDF was estimated by in vitro incubation at 24, 30 and 48 h. Data were analyzed as repeated measures using the MIXED procedure of SAS with fixed effects of treatment and cut. Chemical analysis of experimental silages showed that companion forages reduced ($P < 0.0001$) lignin and crude protein ($P = 0.0006$) concentrations but increased ($P < 0.0001$) NDF and ADF contents compared with the control. Neutral detergent insoluble protein (NDICP), water soluble carbohydrates, total digestible nutrients, and net energy of lactation were similar in all treatments whereas acid detergent insoluble protein (ADICP) was higher ($P < 0.0001$) for control than other treatments. In vitro TTNDFD followed the order ($P < 0.0001$): BSG and BSSG (average 64.7%) > SG (62.8%) > control (56.4%). Evidently, iNDF values were lower ($P = 0.002$) for SG, BSG and BSSG (average 19.5%) than control (28.9%). Digestion rate of potentially digestible NDF was higher ($P < 0.0001$) with companion forages than control. In conclusion, seeding perennial forages with SG, BSG or BSSG may improve chemical composition and NDF digestibility of silages.

Key Words: dairy cow, silage, fiber digestibility

W51 *Staphylococcus* mastitis pathogens are present in milk and horn fly populations. E. K. Luc*, L. G. Schneider, R. T. Trout Fryxell, and G. M. Pighetti, *The University of Tennessee*, *Knoxville, TN*.

Prevention and treatment of mastitis without the use of antibiotics or synthetic products is one of the challenges organic dairies face. Greater understanding of the factors affecting mastitis will aid in developing management programs. Mastitis and fly populations both increase during the summer months, but the relationship between the 2 is unknown in lactating dairy cows housed partly or fully on pasture. Our objective was to identify specific *Staphylococcus* mastitis pathogens (*Staph. aureus*, *Staph. chromogenes*, *Staph. hyicus* and *Staph. agnetis*) in quarter milk samples and horn fly populations. Four organic dairies (n = 67 cows) were enrolled in the study. Sampling occurred from May 2019 through October 2019. Aseptic quarter milk samples were collected, regardless of somatic cell count or clinical status, from the same 15 focal cows on each herd (n = 193 samples) once a month, cultured, and *Staph*. isolates were identified using the API *Staph.* System (bioMerieux Inc., Hazelwood, MO). Live flies from the back and udder area were collected from the same 15 focal cows on each herd (n = 77 cows) every 2 weeks. Flies were pooled (n = 1,702) by farm, date, cow, back or udder, and sex with a max of 15 flies and minimum of 1 fly per pool. DNA was extracted from whole flies using a QIAcube HT (Qiagen, Germantown, MD) and specific *Staph*. species were determined in the fly by PCR and visualized using a QiAxcel (Qiagen). PROC FREQ (SAS 9.4, Cary, NC) was used to determine the frequency of select *Staph*. species in flies and milk samples. To date 384 of 1,702 fly pools have been analyzed. Of those pools tested, 64% were positive for *Staph. aureus*, 47% for *Staph. chromogenes*, 54% for *Staph. agnetis*, and 14% for *Staph. hyicus*. Of the aseptic milk samples collected, 12% (n = 24) were culture positive for *Staph. aureus*, 15% (n = 28) for *Staph. chromogenes*, 0% for *Staph. agnetis*, and 8% (n = 15) for *Staph. hyicus*. All *Staph.* species tested were carried by horn flies and can be potential vectors for intramammary infections and presence in milk. Once all samples are analyzed, the relationship between specific mastitis pathogens from the flies and milk samples will be evaluated.

Key Words: mastitis, horn fly, *Staphylococcus*

W52 Feeding rumen-protected lysine prepartum and postpartum affected plasma metabolites and amino acids in Holstein cows. L. K. Fehlberg*[1], A. R. Guadagnin[1], B. L. Thomas[1], Y. Sugimoto[2], I. Shinzato[2], and F. C. Cardoso[1], [1]*University of Illinois*, *Urbana, IL*, [2]*Ajinomoto Co. Inc*, *Tokyo, Japan*.

During the periparturient period, the demand for protein increases drastically, resulting in a negative protein balance for dairy cows. This experiment was conducted to determine the effects of feeding rumen-protected Lys (RPL; AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) prepartum (0.54%DM of TMR), postpartum (0.395%DM of TMR), or both on plasma AA and metabolites. Seventy-five multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, expected calving date, and body condition score during the far-off dry period were assigned to 1 of 4 dietary treatments in a randomized, complete block design with a crossover of a diet with RPL (L) or without (C). Treatments consisted of TMR topdressed with RPL prepartum and postpartum (LL), with RPL prepartum and without RPL postpartum (LC), without RPL prepartum and with RPL postpartum (CL), and without RPL prepartum and postpartum (CC). Blood samples were collected for plasma AA analyses on −7 ± 0.5, 0 ± 0.5, 7 ± 0.9, and 14 ± 0.9 d relative to calving (DRC). Blood samples were collected for plasma metabolites on −7 ± 0.5, −1 ± 0.6, 0 ± 0.5, 7 ± 0.9, 14 ± 0.9, and 28 ± 0.3 DRC. Statistical analysis was performed using MIXED procedure of SAS. Plasma concentrations of Lys prepartum (69.75μM) were increased ($P < 0.001$) for cows in L compared with C (62.46μM) at −7 DRC. Plasma concentrations of total AA and total dispensable AA prepartum were lower ($P = 0.05$) for cows in L (1810 and 1097 μM, respectively) than C (1924 and 1166 μM, respectively). Postpartum concentrations of Lys in plasma as a percentage of indispensable AA were greater ($P < 0.001$) for cows in L (10.52%) compared with C (9.65%). Plasma concentrations of glucose prepartum were greater ($P = 0.05$) at d 0 (calving) in cows in L (76.14mg/dL) compared with those in C (70.61mg/dL). Plasma concentrations of creatinine phosphokinase were lower ($P = 0.04$) for cows in L (118.0U/L) compared with those in C (131.2U/L) postpartum. In conclusion, cows consuming RPL prepartum increased glucose at calving and increased plasma Lys concentrations.

Key Words: lysine, glucose, transition period

W53 Feeding rumen-protected lysine prepartum enhanced plasma indicators of liver function and increased oxidative burst capacity postpartum in Holstein cows. L. K. Fehlberg*[1], A. R. Guadagnin[1], B. L. Thomas[1], Y. Sugimoto[2], I. Shinzato[2], and F. C. Cardoso[1], [1]*University of Illinois*, *Urbana, IL*, [2]*Ajinomoto Co. Inc*, *Tokyo, Japan*.

A negative energy and protein balance during the periparturient period increases stress in dairy cows and may lead to compromised liver and immune function. This experiment was conducted to determine the effects of feeding rumen-protected Lys (RPL; AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) prepartum (0.54%DM of TMR), postpartum (0.395%DM of TMR), or both on blood metabolites related to immune and liver function. Seventy-five multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, expected calving date, and body condition score during the far-off dry period were assigned to 1 of 4 dietary treatments in a randomized, complete block design with a crossover of a diet with RPL (L) or without (C). Treatments consisted of TMR topdressed with RPL prepartum and postpartum (LL), with RPL prepartum and without RPL postpartum (LC), without RPL prepartum and with RPL postpartum (CL), and without RPL prepartum and postpartum (CC). Blood samples were taken −7 ± 0.5, 0 ± 0.5, 7 ± 0.9, and 14 ± 0.9 d relative to calving (DRC) for detection of phagocytosis and oxidative burst of neutrophils and monocytes in whole blood. Blood samples were also collected for plasma metabolites on −7 ± 0.5, −1 ± 0.6, 0 ± 0.5, 7 ± 0.9, 14 ± 0.9, and 28 ± 0.3 DRC. Statistical analyses was performed using MIXED procedure of SAS. Phagocytosis of monocytes in whole blood postpartum tended to be greater ($P = 0.06$) for cows in C (21.87%) compared with those in L (19.97%). Oxidative burst of monocytes postpartum in whole blood was greater ($P = 0.04$) at 7 DRC for cows in LL and LC (65.64%) compared with those in CC and CL (57.51%) but not at 14 DRC ($P = 0.67$). Plasma concentrations of aspartate aminotransferase, indicative of liver function, were lower ($P < 0.01$) at 14 DRC for cows in LL and LC (73.62 U/L) compared with those in CC and CL (86.81 U/L) but not at 7 or 28 DRC ($P = 0.39$). In summary, consumption of intestinally available Lys prepartum enhances liver function postpartum and increased the oxidative burst capacity of monocytes.

Key Words: lysine, oxidative burst, liver function

W54 Inappropriate use of antimicrobials on dairy farms in midwestern Brazil and their potential risk to public health. A. F. Cruz*[1], D. B. S. Caetano[1], D. C. Silva[2], E. C. Nogueira[3], E. S. Nicolau[4], C. Gebara[4], and L. A. F. Silva[1], [1]*School of Veterinary Medicine and Animal Science, Federal University of Goiás*, *Goiânia, Goiás, Brazil*, [2]*State University of Goiás*, *São Luís de Montes Belos, Goiás, Brazil*, [3]*Maroca Dairy Industry*, *Piranhas, Goiás, Brazil*, [4]*Food Research Center, School of Veterinary Medicine and Animal Science, Federal University of Goiás*, *Goiânia, Goiás, Brazil*.

The notion of one health has been discussed and increasingly demanded in the environmental context, for this reason, care with the use of antimicrobials in all areas of activity is preponderant to minimize the progression of bacterial resistance. Milk is widely consumed by the world population, especially for its nutritional value. However, the presence of chemical residues is a common finding in some countries such as Brazil, preventing direct consumption, and impairing its use on the dairy derivatives products due to the risks to the consumer's health. Therefore, the aim of this work was to highlight the use of antimicrobials in dairy farms in the state of Goiás, in the middle west of Brazil, assessing the potential risk to public health. The antimicrobials use in cattle were analyzed in 132 dairy farms in 33 municipalities in the state of Goiás-Brazil. Farm owners or workers were interviewed by applying a structured questionnaire with questions regarding production parameters and the use of antimicrobials on the property. Responses were analyzed using descriptive statistics and Pearson's chi-squared test (χ^2). Among the 23 active ingredients of antimicrobials mentioned, tetracycline and penicillin were the most used in different rural properties. It was found that 61.4% of the interviewees did not know how to distinguish antimicrobials from other medications such as vermifuges, ectoparasiticides, and vitamins. The milk containing antimicrobial residues was commonly discarded by supplying it to other animals, such as calves, or together with the milking cleaning water, which was not treated. It became evident that in Goiás dairy farms, the use of antimicrobials and the disposal of milk containing residues are commonly carried out inappropriately. Little care was taken with the dose, frequency, and duration of treatments, in addition to the lack of knowledge about the risks of developing resistant microorganisms to the drugs used. Such factors have a high potential for compromising the health of the environment, animals, and man.

Key Words: antibiotic residue, bacterial resistance, public health

W55 Milk fatty acid metrics and their relationship to milk fat and true protein concentrations in tanks from US Jersey herds. H. M. Dann*[1], A. Pape[1], and D. M. Barbano[2], [1]*William H. Miner Agricultural Research Institute*, *Chazy, NY*, [2]*Cornell University*, *Ithaca, NY*.

Our objective was to determine the relationship between milk fatty acid (FA) metrics and concentrations of milk fat and true protein (TP) in tanks from Jersey herds. Bulk tank or tanker milk samples were collected 3 to 7 d per mo for 1 yr from 28 US Jersey herds. Samples were preserved with bronopol and natamycin, refrigerated, and shipped with ice for testing. Samples were analyzed using mid-infrared (MIR) analysis (CombiScope FTIR 300 Hp; Delta Instruments) for fat, TP, de novo (DN: < C16), mixed origin (MO: C16 and C16:1), and preformed (PF: ≥C18) FA, and unsaturation index. Individual sample results were reduced to a monthly mean by herd. A subset of samples (n = 164) were selected, frozen, and analyzed by gas-liquid chromatography (GLC) for individual FA. Individual FA were summed by group and compared with MIR analysis results. Regression and correlation analyses were conducted with R 3.6.2. Fat and TP concentrations increased with increased concentrations of DN, MO, or DN + MO FA. In contrast, fat and TP concentrations were not related to PF FA concentration. Fat ($y = -6.93x + 6.68$; $R^2 = 0.36$) and TP ($y = -3.43x + 4.55$; $R^2 = 0.22$) expressed as a percentage were related negatively to the unsaturation index (x), expressed as double bonds per FA. The unsaturation index was correlated positively ($r = 0.53$) with C18:1 *trans*-10 FA expressed as g FA/100 g milk. There was good agreement between GLC and MIR results expressed as g FA/100 g milk for DN ($r = 0.95$), MO ($r = 0.88$), and PF ($r = 0.77$) FA (Table 1). These findings indicate that MIR analysis is a quick and cost-effective option for determining fatty acid metrics in milk from Jersey herds. Producers and consultants working with Jersey herds should focus on feeding and management practices that promote DN and MO fatty acids in milk to increase tank fat and TP concentrations.

Table 1 (Abstr. W55).

Y variable (%)	X variable (g FA/100 g milk)	Equation	R^2
Fat	DN FA	$y = 2.09x + 2.45$	0.71
Fat	MO FA	$y = 1.53x + 2.21$	0.72
Fat	DN + MO FA	$y = 1.03x + 1.87$	0.84
Fat	PF FA	$y = 0.71x + 3.68$	0.08
TP	DN FA	$y = 1.30x + 2.14$	0.66
TP	MO FA	$y = 0.66x + 2.50$	0.33
TP	DN + MO FA	$y = 0.53x + 2.11$	0.53
TP	PF FA	$y = 0.04x + 3.60$	<0.01

Key Words: milk fat, milk protein, de novo fatty acids

W56 A survey of manure management in Louisiana dairy farms. V. R. Moreira*[1], B. D. LeBlanc[1,2], C. Franze[1,2], E. M. Mackey[1,3], and C. A. Njombwa[1,4], [1]*Louisiana State University Agricultural Center*, *Baton Rouge, LA*, [2]*Louisiana State University Sea Grant*, *Baton Rouge, LA*, [3]*Washington State University*, *Puyallup, WA*, [4]*Lunyangwa Agricultural Research Station*, *Mzuzu, Malawi*.

The number of Louisiana dairy farms has declined at rates >8%/yr, while cow numbers decreased by >70% in the last 15 years. Despite the decline in dairy farms, considerable concerns about manure spills and water contamination remain. The objectives of this study were to assess current dairy manure management in Louisiana. The survey was developed and approved by a stakeholder committee including dairy farmers, state and federal agencies, non-governmental organization and university personnel. This study summarizes part of a survey evaluating ownership characteristics, current management practices, and current and potential assistance programs. A study questionnaire was collected by trained personnel and data were managed using REDCap electronic applications hosted at Louisiana State University. The response rate was 41.7% among dairy farmers holding Louisiana Department of Health permits. Nearly 83% of farms surveyed were operated privately or in family partnership. Most dairies (93% of respondents) were located on Lake Pontchartrain watershed and most had 200 cows or fewer (88.4%). Liquid was the prevailing form of manure flushed or washed (95%) off the milking parlor and waiting area (97.7%), where cows spent an average of 4.9 h/d (4.7 h/d in summer and 5.1 h/d in winter). Cows spent most of the remainder of the day (18 h) on pasture. Liquid manure was managed in lagoons designed to hold effluent over multiple years: more than 2/3 of those farms had lagoon effluent pumped out at least once in the previous 5 years. Nearly 1/3 of the participating dairies also collected solid manure and at least half of the respondents indicated they had solid separation technologies such as sand traps and settling basins. Few of those operations had solid treatment (7% had composting facilities) and most preferred to store solid manure directly in the solid spreader. Pastures (86%) and hay fields (46.5%) were common sites for wastewater application using stationary equipment like pumping stations and risers (80%). The results of this survey provide guidance for the development of assistance programs that are better suited to attend the needs of grazing dairy operations in the US Southeast.

Key Words: manure management, survey, dairy

W57 Effects of particulate matter on health and production of dairy cattle. A. A. Anderson*, P. Rezamand, A. Ahmadzadeh, and A. L. Skibiel, *University of Idaho*, *Moscow, ID.*

Seasonal wildfires affect a large portion of the country and are expected to increase in size and severity in the coming years due to drier conditions and hotter summers brought on by global climate change. Because wildfires cause severe episodic reductions in air quality, the health and production of livestock in open-air housing may be adversely affected. The objective of this study was to examine associations between increased fine particulate matter during the wildfire season and incidence of disease and changes in production of Holstein dairy cows. Health records for lameness, mastitis, pneumonia, metritis, displaced abomasum, retained placenta, abortion and death were collated from a large dairy (>5,000 head) in Washington and monthly milk production records were obtained from a small farm in Idaho (~100 head milking). Air quality data from a monitoring site 14 km from the Washington farm was extracted from archived data maintained by the Washington State Department of Ecology. Idaho air quality data recorded from a monitoring site 5.9 km from the Idaho farm were obtained from the Idaho Department of Environmental Quality. Air quality records, health records, and production (milk yield and composition) data were collated from May through September (encompassing the Pacific Northwest wildfire season) across a 5-year period (2015–2019). Peaks in $PM_{2.5}$ observed were concurrent with known wildfire events in both states. Data were analyzed using a generalized linear model and a negative binomial distribution. Lags of up to 2 weeks were included in the models to account for potential delays in the effects of particulate matter exposure. $PM_{2.5}$ was significantly positively associated with general illness ($P = 0.025$), mastitis ($P < 0.0001$) and calf deaths ($P = 0.003$). No significant relationships between particulate matter and milk yield or components were found; however, the broad time scale of monthly milk records may have precluded detection of any effects. Our data indicate that exposure to high particulate matter is associated with adverse health outcomes in dairy cattle.

Key Words: climate change, $PM_{2.5}$, air quality

W58 Organic additives in diet of dairy cows can reduce the conventional antiparasitics to control ticks. L. Ferreira*[1], A. Daurea[1], L. Bertelli[1], A. Nascimento[2], M. Lima[2], and R. Mendonça[2], [1]*Premix*, *Ribeirão Preto, SP, Brazil*, [2]*Universidade de Franca*, *Franca, SP, Brazil.*

The inclusion of fatty acids as additives organics in diet of the animals can to improve the immune response of animals, reduces an application of carapaticidal agents and makes the practice sustainable. This study was to evaluate the action of an organic additive as an aid in controlling the tick *Rhipicephalus* (*Boophilus*) *microplus.* 20 dairy cows was divided into 2 experimental groups. The cows were stratified based on the teleogen count present on the left side of each naturally tick-infested animal, using an average of 3 counts on consecutive days before treatment. The treated group received daily the organic additive Fator P and the control received the same mineral supplementation but without the additive. Fator P is an organic additive composed to: aminoacids (lysine, methionine, and tyrosine), the choline, minerals, probiotics and fatty acids. To evaluate the action of the additive, counts 3, 5, 7, 14, 21, 28, 57, 86, 115, 144, 172, 200, 227, 254 and 281 d after the beginning of treatment were performed. To avoid ticking overcrowding, animals that had counts greater than 30 teleogins were treated with conventional antiparasitics. The experimental showed efficacy was greater than 50% in 5 d out of 15 evaluated, with a statistically significant difference between experimental groups in 6 of these assessment days (Two Way ANOVA and posttest Bonferroni). The control group received conventional treatment 18 times more than the treated. There was a reduction of 64.28% in the number of treatment in animals supplemented with the organic additive, thus providing an auxiliary action in the control of ticks.

Table 1 (Abstr. W58). Means and standard deviation of tick count

Experimental day	Control	Treated
3	6.0 (±3.9)	5.1 (±5.5)
5	0.0 (±0.0)	0.0 (±0.0)
7	0.0 (±0.0)	0.0 (±0.0)
14	0.0 (±0.0)	0.0 (±0.0)
21	2.4 (±1.9)	1.9 (±1.0)
28	1.2 (±1.7)	3.0 (±3.8)
57	8.8 (±6.3)	9.8 (±5.9)
86	11.4* (±7.2)	26.7* (±23.7)
115	24.2** (±11.5)	7.6** (±2.1)
144	27.0 (±17.9)	16.5 (±10.7)
172	25.5* (±15.0)	10.7* (±9.9)
200	24.3* (±18.9)	9.0* (±9.7)
227	34.8*** (±15.5)	8.1*** (±9.6)
254	22.8* (±14.2)	7.9* (±11.1)
281	16.6 (±14.9)	9.1 (±12.3)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Key Words: animal nutrition, tick efficacy, alternative control

W59 Effects of heat stress and OmniGen-AF on physiological and inflammatory responses of mid-lactation dairy cows to intravenous lipopolysaccharide infusion. T. N. Marins*[1], J. Gao[1], Q. Yang[1], R. M. Binda[1], C. M. B. Pessoa[1], R. M. Orellana[1], J. K. Bernard[1], M. Garcia[2], D. J. McLean[2], J. D. Chapman[2], D. J. Kirk[2], and S. Tao[1], [1]*University of Georgia*, *Tifton, GA*, [2]*Phibro Animal Health Corp*, *Teaneck, NJ.*

Our aim was to evaluate hormonal and cytokine responses of mid-lactation Holstein cows fed OmniGen-AF (OG; Phibro Animal Health) or not with or without evaporative cooling to an intravenous lipopolysaccharide bolus (ivLPS) during summer. Multiparous cows (n = 60) were blocked by parity and DIM, and randomly assigned to 4 treatments (TRT) in a 2 × 2 factorial arrangement with 2 environments (E): cooled (CL) using fans and misters or non-cooled (NC), and 2 top-dressed feed additives (D, 56 g/d): OG or placebo as control (CON). All cows were cooled and fed respective diets for 60 d before experiment. At d 52 of the experiment, a subset of cows (n = 6/TRT) was iv-injected with LPS (0.20 μg/kg of BW). Blood collected at −1, −0.5, 1, 2, 3, 4, 6, 8, 12, 18 and 24 h relative to ivLPS were analyzed for cortisol (COR), prolactin (PRL), tumor necrosis factor (TNF) α and interleukin (IL) 10. From d −1 to 2 of ivLPS, daily DMI, milk yield (MY) at each milking, and vaginal temperature (VT, every 5 min) were recorded. Relative to CL, VT of NC cows had similar peak at 3 h, but remained greater before and after ivLPS (E × Time [T]: $P < 0.01$). ivLPS reduced DMI and MY (T: $P < 0.01$), without TRT × T interactions ($P > 0.1$). Typical ivLPS acute responses on peak concentrations of COR, PRL, TNFα and IL10 were respectively observed at 3, 1, 1, and 1 h after ivLPS (T: $P < 0.01$). CL-OG cows had greatest COR area under the curve (AUC) from 0 to 3 h (D × E, $P = 0.07$) than other TRT. Although CL-OG cows had greatest ($P = 0.02$) cortisol 1h after ivLPS, NC-OG reached similar peak as CL-OG at 3 h, which was greater ($P = 0.03$) than both CON TRT (D × E × T, $P = 0.01$). For CON cows, PRL AUC from 0 to 3 and 0–6 h were lower in NC cows; whereas both OG TRT had similar AUC, which did not differ from both CON TRT (D × E, $P \leq 0.04$). OG cows tended to have greater ($P = 0.09$) IL10 AUC from 0 to 3 h than CON. In conclusion, OG increased acute hormonal response to ivLPS in NC cows (greater COR, intermediate PRL), and enhanced IL10 secretion regardless of E, suggesting OG enhances acute response to ivLPS while promoting a faster resolution.

Key Words: heat stress, OmniGen, inflammation

W60 The use of OmniGen-AF in New Zealand dairy herds: An observational study.

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Six dairy farms in New Zealand were selected based on data recording and the availability of feed pad systems for an observational study. The health and milk production were measured on grazing animals that received OmniGen-AF (OG), a nutritional supplement designed to support a healthy immune function in cattle. All herds were seasonal, spring calving herds. A partial mixed ration (PMR) was delivered with a mixer wagon onto a concrete feed pad. OmniGen-AF was included in the PMR at an estimated daily feeding rate of 9 g OG / 100 kg BW from 3 weeks before calving to 100–160 DIM. Data on milk production and bulk tank somatic cell count (BTSCC) was collated from the calving start date to 160 DIM. The incidence rates of lameness and mastitis were estimated from each farm's records sheet. The diagnosis of these diseases was made directly by on-farm personnel. Milk solids production, BTSCC and health outcomes from the current season (CUR, July–December, 2018) and the average of the 2 previous seasons for milk and BTSCC (PREV, July–December, 2016, 2017) or last season (July–December, 2017) for health outcomes, were evaluated. Milk solid production was analyzed using a paired *t-test* analysis. Beta-regression was used for analyzing mastitis and lameness rate, and binomial regression was used for BTSCC. On average, milk solids production increased by 6% (1.75 vs. 1.65 kg/d, for CUR vs PREV, respectively, $P = 0.04$). The BTSCC was not different ($P = 0.34$) but numerically reduced by 18% across all farms in CUR vs. PREV. The mastitis incidence was not different (5.8 vs. 6.8, for CUR vs PREV, respectively, $P = 0.69$) but lameness rate was decreased ($P = 0.04$) by 62% (7.9 vs. 15.9 for CUR vs PREV, respectively). Differences on other health indicators were not consistent among farms. The observed reductions in lameness, and increased milk solids production in CUR vs. PREV, suggest that OmniGen supplementation may have assisted in improving health and productivity in these New Zealand farms

Key Words: OmniGen-AS, New Zealand, immunity

W61 Differences in methane production, yield, and intensity and its effects on metabolism of dairy heifers.

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The effects of divergent phenotypic classification in crossbreed Holstein × Gyr dairy heifers for methane emissions in relation to performance, digestibility, energy and nitrogen partition, blood metabolites and temperature of body surface were evaluated. Thirty-five heifers were classified as high and low emission for CH_4 production (g/d), yield (g/kg dry matter intake) and intensity (g/kg average daily gain). Digestibility was evaluated by total collection of feces and urine. Gas exchanges were obtained in open-circuit respiratory chambers. A completely randomized design was used and divergent groups was compared by Fisher's test. No differences were found in intake traits between groups of CH_4 production and intensity. The low yield group had higher intake (6.20 vs. 7.02 kg/d, $P = 0.003$). For digestibility and temperature at different body sites were no differences between variables. High production group had higher energy losses as methane (1.95 vs. 1.68 Mcal/d, $P = 0,01$) and heat production (13.0 vs. 11.5 Mcal/d, $P < 0.001$). Low intensity group had higher digestible energy intake (19.8 vs. 22.5 Mcal/d, $P = 0.04$), energy balance (4.78 vs. 8.02 Mcal/d, $P = 0.02$) and ratio between metabolizable and digestible energy (0.87 vs. 0.89, $P = 0.01$). Urinary nitrogen was 14.3% lower for low production group. There was a difference between methane yield divergent groups for nitrogen intake (170 vs. 188 g/d, $P = 0.001$), digestible (133 vs. 150 g/d, $P = 0.003$) and retained (27.7 vs. 54.3 g/d, $P = 0.001$). Energy and nitrogen partitioning traits are correlated with the animals divergent for methane production and yield. The low production group presented lower blood insulin concentration (39.8 vs. 30.6 μIU/mL). It was not possible to identify divergent animals for CH_4 emission using the infrared thermography technique.

Key Words: calorimetry, greenhouse gas, nitrogen utilization

W62 Effects of virginiamycin on milk yield and ruminal pH of lactating dairy Holsteins cows.

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An on-farm study investigated the effects of feeding 500 mg/d of virginiamycin (VM) for 74 d on rumen pH, feed intake, and milk yield (MY) of lactating Holstein cows in Mexico. Cows of similar DIM were randomly split into 2 treatments in 2 pens: control (C) (n = 90) and VM (n = 93), then 15 cows from each treatment were randomly selected to receive indwelling sensors to measure ruminal pH and temperature wirelessly. Measurements included cow ruminal pH every 10 min for 40 d, daily pen ration DMI, daily cow MY, and weekly pen milk fat and protein contents. Daily energy-correct MY (ECMY) was computed by interpolating weekly milk fat and protein contents of each treatment to daily contents and adjusted for 3.5% fat and 3.2% protein. Statistical analyses were conducted as repeated measurement design (RMD) for ECMY and ruminal pH, and one-way ANOVA for DMI. The area under the curve (AUC) and time under the curve (TUC) for ruminal pH below 5.4, 5.8, and 6 were computed daily for each cow, using spline interpolation with the Forsythe, Malcolm, and Moler method. Weekly ruminal pH was analyzed using DMI as a covariate. VM-treated cows produced 5.5 kg/d (40.6 vs. 35.1; $P < 0.01$) and consumed 2.6 kg DM/d (24.0 vs. 21.4; $P < 0.01$) more than C cows, increasing the milk-to-feed ratio by 3% (1.69 for VM and 1.64 for C; respectively). The RMD analysis indicated that VM cows had lower minimum ruminal pH than C cows (5.37 vs. 5.49; $P < 0.01$), but average and maximum ruminal pH did not differ ($P > 0.09$). The AUC analysis confirmed the RMD findings but also indicated that the AUC and TUC were not different ($P > 0.12$) between VM and C cows for ruminal pH below 6, 5.8, 5.6, or 5.4. The ruminal pH may have been affected by the 12% greater DMI of the VM compared with the C cows. When ruminal pH was adjusted for weekly DMI and compared at the C DMI (20.4 kg/d), the minimum ruminal pH was no longer statistically different ($P > 0.07$), but the average ruminal pH tended to be greater for VM-treated cows. The increased milk-to-feed ratio for VM-treated cows suggests VM might have improved rumen health or other metabolic parameters that need to be studied

Key Words: virginiamycin, acidosis, production

W63 Using activity and rumination data to early predict anaplasmosis in dairy calves. V. A. Teixeira[1], G. M. Souza[2], L. D. Ferreira[1], T. R. Tomich[3], T. Bresolin*[4], M. M. Campos[3], A. M. Q. Lana[1], S. G. Coelho[1], J. A. G. Silveira[1], A. U. Carvalho[1], E. J. Facury Filho[1], L. G. R. Pereira[3,4], and J. R. R. Dorea[4], [1]*Universidade Federal de Minas Gerais*, *Belo Horizonte, MG, Brazil*, [2]*Universidade Federal de Lavras*, *Lavras, MG, Brazil*, [3]*Brazilian Agricultural Research Corporation – Embrapa*, *Juiz de Fora, MG, Brazil*, [4]*University of Wisconsin*, *Madison, WI*.

Bovine anaplasmosis causes large economic losses in dairy cattle production systems worldwide and is commonly detected through rectal temperature, blood smears under a microscope, and packed cell volume (PCV). Such methodologies are labor, costly, and difficult to apply in large scale operations. The objective of this study was to investigate the feasibility of using activity and rumination data retrieved from the SCR Heatime HR collar to identify dairy calves with anaplasmosis. Thirty-four calves with an average age of 119 ± 15 d (148 ± 20 kg of BW) were challenged with 2 × 10^7 erythrocytes infected with UFMG1 strain (GenBank no. EU676176) isolated from *Anaplasma marginale*. After the challenge-exposure, animals were monitored every day by assessing PCV. A PCV critical level of 16 ± 3.6% was used as a threshold to classify the animals as sick (0 d) and to initiate an enrofloxacin treatment. Two-time series (TS) were built using the activity and rumination data sets. The first TS (TS1) included the last sequence of 8 d (−8 d to −1 d) preceding the PCV critical level and the second TS (TS2) comprised a sequence of 8 d randomly selected in a window from −50 to −15 d before the PCV critical level. TS2 was randomly selected within the animal to ensure a sequence of days in which PCV was considered normal (32 ± 2.4%). Therefore, TS1 and TS2 were assumed to be a sequence of days resulting in critical and non-critical PCV levels, respectively. A Recurrent Neural Network (RNN) approach implemented in Keras R package was used to analyze activity and rumination data sets. To validate the models, leave-one-out cross-validation was performed by removing a pair of time series (TS1 and TS2) per animal at a time. RNN predicted anaplasmosis animal infection from rumination data with accuracy, sensitivity, specificity, positive predictive value, and negative predicted value of 84, 76, 91, 90, and 79%, respectively, whereas for activity these values were 74, 71, 76, 75, and 72%, respectively. In conclusion, activity and rumination data from wearable sensors can potentially be used as early predictors of bovine anaplasmosis in dairy calves.

Key Words: precision dairy farming, rickettsia, tick fever

W64 Effect of heifers rearing manner during liquid milk nutrition on their growth and maze behavior after 12 months. J. Broucek*, M. Uhrincat, P. Kisac, and A. Hanus, *National Agricultural and Food Centre*, *Luzianky, Slovakia*.

The objective of this study was to test the hypotheses that heifers growth and behavior after 12 mo (M) are affected by feeding/housing before weaning. Fifty-one Holstein heifers were assigned to 1 of 3 rearing treatments: SM, n = 18, pen with mother (milked from d 2) to d 21, suck a mother's udder 3 times daily, then group pen (6 kg milk daily, bucket with nipple); SN, n = 16, after 3 d with own mother in pen with nursing cow, calves could drink at any time, number of calves per nursing cow determined according to milk yield (6 kg milk per d.calf^{-1}); H, n = 17, after having nursed their dams in individual pen for 24 h in hutch to d 56 (bucket with nipple, milk replacer (MR) 6 kg daily), then loose housing pen to weaning (bucket with nipple, MR 6 kg daily). Group SM allowed 21 d suckle, 63 d bucket fed; SN allowed 84 d suckle; H allowed 1 d suckle, 83 d bucket fed. From d 2 until weaning the heifers were fed concentrate and alfalfa hay ad libitum. The weaning was at d 84. After weaning, all heifers were kept in age-balanced groups in bedded pens with the same ration. The growth of live body weight (LBW) was recorded. The maze behavior was tested in the 12th and 19th month (M) of the age. Heifers solved 6 tasks during 3 d in the 8 × 14 m facility. The time of speed of traversing the maze was recorded. The data were analyzed using a General Linear Model ANOVA. The highest LBW was recorded in SN and the lowest in H (SM 336 ± 42 kg, SN 362 ± 46 kg, H 328 ± 41 kg, $P < 0.01$). The ADG from d 360 to 570 were 0.89 ± 0.29 kg (SM), 0.86 ± 0.31 kg (SN), 0.72 ± 0.27 kg (H) ($P < 0.05$). At the 12 and 19 M, SN crossed the maze fastest and H slowest. The times of all tests differed significantly (12 M: SM 1220 ± 370 S, SN 892 ± 424 s, H 1345 ± 490 s, $P < 0.05$; 19 M: SM 1198 ± 377 S, SN 909 ± 408 s, H 1290 ± 390 s, $P < 0.05$). The results indicate that the method used to rear heifers may have a significant impact on their later growth and behavior.

Key Words: rearing, growth, behavior

W125 Effect of a low-DCAD, high-calcium acidogenic ration on hypocalcemia and health outcomes in New Zealand farms. B. J. McKay[1], N. Chrystal[2], K. Zanzalari[3], and M. Gorocica*[3], [1]*Dairy Production Systems Ltd., Hamilton, New Zealand*, [2]*Complete Feed Solutions Ltd., Auckland, New Zealand*, [3]*Phibro Animal Health Corporation, Teaneck, NJ*.

An observational study was conducted to assess the effectiveness of a low DCAD, high calcium ration in reducing hypocalcemia (HC) and HC-associated disorders under New Zealand feeding conditions. Four farms with high incidence rates of clinical milk fever (5.5% over the last 3 yr) were selected. Results from the current (CUR) vs. the previous (PREV) calving seasons were contrasted using β-regression with marginal means comparison. In both CUR and PREV seasons, a concentrate mix was formulated to complement estimated forage intake to meet or exceed NRC (2001) requirements. DCAD and Ca levels were the main differences between concentrate offered in CUR vs. PREV seasons. For PREV, the total ration DCAD ranged from −47 to +92 vs −100 to −150 mEq/kg DM in the CUR season. Total Ca supplied was estimated at 85 g vs. 180 g/d in PREV and CUR, respectively. The concentrate mix was offered during the last 21–28 d of gestation. Urine pH was measured weekly. Target range for urine pH was 5.5–6.0. If urine pH was off target, the amount of concentrate mix offered was adjusted accordingly. A 69% reduction ($P < 0.01$) in the incidence rate of clinical HC was observed (5.4% vs. 1.9% for PREV and CUR, respectively). Calving assists (5.1 vs. 2.8%), retained placenta (4.0 vs. 2.0%) and total peri-calving disorders (18.4 vs 7.4%), were all numerically reduced in the CUR vs. PREV seasons but variation across farms and the relatively small number of farms enrolled in the study prevented reaching statistical significance (all $P > 0.10$). In conclusion, health outcomes during the CUR season suggest a low DCAD, high calcium ration may be an effective tool to manage hypocalcemia and other related issues under grazing conditions in New Zealand

Key Words: hypocalcemia, DCAD, New Zealand

Ruminant Nutrition: General 3

W65 Health and growth in Holstein calves fed blends of yeast products, phytogens, and direct-fed microbials in milk replacer and starter. K. N. Brost*[1], D. M. Paulus Compart[2], K. N. Russo[2], and J. K. Drackley[1], [1]*University of Illinois Urbana Champaign*, *Urbana, IL*, [2]*Land O'Lakes Inc*, *Arden Hills, MN*.

The objective of this study was to determine if additives fed in milk replacer (MR) and starter could enhance health and growth of Holstein calves through 8 wk of age. The experiment was a randomized complete block design with 32 male calves per treatment, purchased from a commercial dairy when ≤ 3 d old. Calves were housed in individual hutches bedded with straw. Calves were fed MR (22% CP, 20% fat) at 0.6 kg (DM) daily, and starter for ad libitum intake. Treatments included: control MR and starter with no additives (CON); a commercially available blend of phytogens and yeast extract (Surmount, PMI, Arden Hills, MN) in MR and a blend of yeast products and phytogens (Victant, PMI) in starter (CTP); and an experimental blend of direct-fed microbials and phytogens (EXP-MR) in MR and a blend of yeast products, phytogens, and direct-fed microbials (EXP-S) in starter (CBP). Intakes of MR, starter, and water were recorded daily. Measurements of BW, body length, withers height, hip height, and hip width were recorded weekly. Calves were weaned on d 49. Health data were analyzed using GLIMMIX and FREQ procedures, and growth and intake data were analyzed using the MIXED procedure in SAS. The number of days calves were medicated differed among treatments ($P = 0.02$), with CTP medicated the least (0.69 d/calf) compared with CON and CBP (1.58 and 1.69 d/calf, respectively). Electrolyte administrations tended ($P = 0.09$) to be more frequent for CTP than CON or CBP. Instances of fecal scores > 2 (4-point scale) were not different ($P = 0.33$). Respiratory scores showed a pattern of fewer events for CON and CTP (15 and 17 events, respectively) than for CBP (34 events). Nasal discharge showed a similar numerical difference among treatments. Intakes of MR, starter, and water did not vary among treatments ($P > 0.41$). No differences were detected in BW or other growth measurements, except for hip width that tended to be greater for CBP than CON ($P = 0.071$). To conclude, CTP improved health outcomes compared with CON and CBP but did not improve growth.

Key Words: phytogen, direct-fed microbial, calf

W67 The use of reduced levels of organic minerals in diets for transition dairy cows. G. Silva[1], M. Dias[1], N. Grigoletto[1], T. Del Valle[2], A. Nunes[1], P. Curti[1], P. Vittorazzi Junior[1], T. Silva[1], C. Takiya[3], J. Pettigrew[4], A. Koontz[5], L. Costa e Silva[5], and F. Rennó*[1], [1]*University of São Paulo*, *Pirassununga, SP, Brazil*, [2]*Unipampa*, *Itaqui, RS, Brazil*, [3]*Kansas State University*, *Manhattan, KS*, [4]*James Pettigrew Consulting*, *Nicholasville, KY*, [5]*Alltech Inc*, *Nicholasville, KY*.

The use of organic sources, especially trace minerals, can be important during the transition period for dairy cows. The objective of the study was to evaluate the effect of reduced levels of organic trace minerals on milk yield and composition, dry matter intake and trace mineral levels in plasma, colostrum and liver of transition dairy cows. Twenty-four Holstein cows were assigned to 12 blocks, according to the body condition score, parity, and milk yield from the last lactation, for the following treatments: 1) Control (CON), following the NRC (2001) guidelines for Se, Zn, Cu, Co, and Mn as inorganic form (sulfates) and 2) Proteinated minerals (PM) and Se yeast (PM) (Bioplex Co, Cu, Mn, Zn, and Sel-Plex, Alltech Inc., Nicholasville, KY), at 50% of the NRC (2001) recommendation for Zn, Cu, Co, and Mn; Se was included at the NRC (2001) recommendation for both treatments. Treatments were provided from 4 weeks before the expected calving date through 8 weeks of lactation. Milk production and composition and dry matter intake were evaluated weekly. The determination of micro minerals (Se, Zn, Cu, Co, and Mn) in plasma and liver were measured −30 d before calving, day of calving, and 56 d postpartum. Data were analyzed using the MIXED procedure of SAS (2001). The PM increased ($P = 0.006$) milk yield (27.7 vs 30.9kg/d), protein ($P = 0.006$) (0.890 vs. 0.976 kg/d) and lactose ($P = 0.008$) (1.33 vs. 1.46 kg/d). PM tended to decrease ($P \leq 0.075$) the concentration of hepatic Cu (55, 7 vs. 48.3 mg/kg) and increased ($P = 0.016$) plasma Se concentration (105 vs 119 µg/L). Dry matter intake was higher ($P = 0.044$) for animals in the PM diet (18.8 vs. 21.1 kg/d). In colostrum, PM treatment increased ($P = 0.019$) Se concentration (48.5 vs. 71.3 µg/L). Therefore, the inclusion of proteinated trace minerals (Zn, Cu, Co, and Mn) at 50% of NRC recommendations increased dry matter intake, and consequent milk yield, protein, and lactose contents in the milk without affecting trace minerals levels in the liver.

Key Words: milk production, liver biopsy, mineral concentrations

W68 The effect of a sanguinarine supplementation on feed intake, weight gain, hematology, and serum chemistry of calves. H. K. J. P. Wickramasinghe*[1], N. Stepanchenko[1], C. A. Kaya[2], J. V. V. Silva[1], S. Bas[3], F. R. B. Ribeiro[3], and J. A. D. R. N. Appuhamy[1], [1]*Department of Animal Science, Iowa State University*, *Ames, IA*, [2]*Dicle University*, *Diyarbakir, Turkey*, [3]*Phytobiotics North America LLC*, *Cary, NC*.

Plant-derived alkaloids such as sanguinarine are found to be beneficial in improving growth performance, and the immunity of animals. The objective of the present study was to examine the effects of a *Macleaya cordata* extract (MCE), standardized to contain 1.5% of sanguinarine on DMI, weight gain, weight gain: DMI (FCE), hematology, and serum chemistry of young calves. Forty (20 male and 20 female) crossbred dairy-beef calves of 6.0 ± 2.0 d of age were used. Calves were matched for age, sex, and BW and assigned to 4 MCE doses; 0 (CTL), 2.0 and 4.0 (D1), 5.0 and 10 (D2), and 10 and 20 (D3) g/calf/d in pre- and post-weaning, respectively. A milk replacer (MLR, 12% solid in 6.0 L/d) was bottle-fed until calves were 49 d of age. MCE was fed for 90 d mixing daily in MLR pre-weaning and top-dressing on a starter feed post-weaning. Body weight, hematology and serum chemistry were measured on 0, 45 (around weaning), and 90 d. The hematology and serum chemistry analyses were performed in a GLP-certified laboratory (Quality Veterinary Laboratory, Davis CA). The treatment effects were analyzed with a model including fixed effects of treatment (the MCE dose), sex, age, and random effect of calf. The baseline measurements of hematology and serum chemistry were included as covariates in the model. The association of MCE with each response variable was tested using linear and quadratic orthogonal contrasts. The DMI (MLR + starter feed) of pre-weaned calves linearly ($P < 0.001$) increased with MCE dose. The MCE dose had a quadratic relationship with starter intake post-weaning ($P = 0.021$). Weight gain and FCE did not change with MCE dose pre- or post-weaning. No hematology parameter changed with MCE dose ($P > 0.050$). All serum chemistry parameters except creatinine were similar ($P > 0.050$) among MCE doses. Creatinine linearly increased ($P = 0.049$) with MCE dose. Overall, MCE supplementation at 10 (pre-weaning) or 20 (post-weaning) g/d appeared to be safe in calves. Performance improvements for MCE could be captured with a larger sample size.

Key Words: sanguinarine, feed intake, calves

W69 Effect of *Pogostemon cablin* extract on proliferation and differentiation of 3T3-L1 preadipocytes and improve insulin resistance. H. Y. Gao, C. Xu*, Y. Z. Cui, and R. X. Chang, *Heilongjiang Bayi Agricultural University*, *Daqing, Heilongjiang, China*.

To study the effect of *Pogostemon cablin* on the promotion of 3T3-L1 preadipocytes differentiation and the regulation of glucose and lipid metabolism in adipocytes. Different concentrations of *Pogostemon cablin* (0.5, 1.0, 2.5 and 5.0 mg/mL) were added into cell culture plate on the first day, On the eighth day, lipid droplets accumulated in cytoplasm of the differentiated preadipocytes were observed by red O staining and quantified by colorimetry. ELISA method was used to detect intracellular triglyceride

(TG)levels. Western blot was used to determine PPARγ2 protein expression. Tetramethylazazole blue (MTT) method was used to analyze the effect of pogostemon cablin on the proliferation and differentiation of 3T3-L1 cells. Glucose oxidase-peroxidase method was used to detect glucose concentration in mature adipocytes.The results show that Pogostemon cablin can inhibit the proliferation of 3T3-L1 preadipocytes and promote the differentiation of cells to mature adipocytes ($P < 0.05$).With the increase of pogostemon cablin concentration, gradually increased lipid storage, gradually increased TG level ($P < 0.05$).Compared with the induced differentiation group, the expression of PPARγ2 protein in pogostemon cablin group (0.5, 1.0, 2.5 and 5.0 mg/mL)was significantly increased ($P < 0.05$). Compared with the insulin-resistant mature 3T3-L1 adipocyte group, it was found that pogostemon cablin can significantly promote glucose consumption and improve insulin resistance in mature adipocytes ($P < 0.01$). Pogostemon cablin can promote the differentiation of 3T3-L1 preadipocytes, promote the accumulation of intracellular lipid droplets and regulate the metabolism of glucose and lipids, thereby improving insulin resistance.

Key Words: 3T3-L1 preadipocytes, differentiation, insulin resistance

W70 Models to predict enteric methane emissions from cows fed different forage sources. R. Manconi[1], A. S. Atzori*[1], and J. A. D. R. N. Appuhamy[2], [1]*Dipartimento di Agraria, University of Sassari*, *Sassari, Italy*, [2]*Iowa State University*, *Ames, IA*.

Addressing the diversity in forage sources is a key requirement of developing region-specific models to predict methane emissions (CH_4) from dairy cattle. This study was aimed to develop empirical models to predict enteric CH_4 from dairy cows fed different forage sources. A literature search was conducted for trials with enteric CH_4 measurements (g/cow/d) of European dairy cows, which were published from 2000 to 2019. The database had 41 studies providing 195 treatment means of the CH_4 of cows fed: 1) corn silage (CS, n = 37), 2) grass silage (GS, n = 66), 3) corn silage and grass silage (CGS, n = 69), and 4) pasture (PST, n = 23). Data were divided to 4 subsets based on the forage source. The models to predict CH_4(g/cow/d) were developed separately for each forage source with a random-effect meta-analysis approach using the *metafor*package in R software. To avoid multicollinearity issues, 3 separate model development schemes including DMI (kg/d), GEI (MJ/d), or milk yield (MY, kg/d) with dietary CP, NDF, and EE (% of DM), milk protein (MPr, %) and milk fat (MFt, %), and BW (kg) were employed within each forage source. Models were evaluated using data used for model development. Following models having lowest root mean square prediction error as a % of mean observed value (RMSPE) were ranked best within each forage source. CS: $CH_4 = -431.7 + (1.4 \times GEI) + (23.2 \times CP) - (13.7 \times EE) - (6.2 \times NDF) + (39.8 \times MFt)$ GS:$CH_4 = -282.0 + (11.9 \times MY) + (2.9 \times CP) - (6.6 \times EE) + (4.9 \times NDF) - (0.04 \times BW) - (44.8 \times MFt)$ CSG: $CH_4 = 171.6 + (8.7 \times DMI) - (2.9 \times CP) - (21.1 \times EE) + (0.2 \times BW) - (26.7 \times MPrt) + (12.3 \times MFt)$ PST: $CH_4 = 271.1 - (2.8 \times MY) - (3.2 \times CP) + (31.2 \times EE) - (5.3 \times NDF) + (0.6 \times BW)$ The best model in CS, GS, CGS, and PST predicted the observed CH_4 well as evident by low RMSPE (18.6, 11.5, 8.6, and 13.3%, respectively), a major proportion of which (95, 95, 99, and 86%, respectively) was due to random variability of data. This study provides a set of models that can be used predict accurately CH_4 from dairy cows fed wide variety of forages using routinely available information. Models however need to be evaluated possibly with an independent data set to draw firmer conclusions on their predictive power.

Key Words: meta-analysis, grass, Europe

W71 Lactational performance in dairy cows receiving a rumen-protected B-vitamin blend during lactation: Meta-analysis of 65 studies. E. Evans[2], H. Leclerc*[1], and E. Santin[1], [1]*Jefo*, *St-Hyacinthe, QC, Canada*, [2]*Technical Advisory Services*, *Bowmanville, ON, Canada*.

The objective of this study was to evaluate the impact of a supplemental rumen protected blend of B vitamins providing essential vitamins pantothenic acid, pyridoxine, biotin, folic acid and vitamin B_{12} (Jefo, Canada) on milk, fat and protein yield in cows past peak lactation. Data from 65 on farm studies (50 double reversal in which the test product was supplied in addition to the currently fed diet in the center period and 15 single crossover design) conducted between 2005 to 2015 were analyzed using Meta-Essentials Software (Creative Commons, 2017). Within each study, cows included were over 45 d in milk at first milk test, and available for all milk collection periods (minimum 28 d). All herds participated in registered monthly recording services and all were Holstein herds. A total of 9839 cows were represented in the 65 studies from 8 countries, with an average herd size of 151.4 + 160.2. Effect size was calculated for milk, fat and protein yield based on the treatment differences and standard errors (SE) from the individual trials. Mean effect size, SE and 95% confidence intervals (CI) are given in Table 1. A subgroup analysis was conducted to determine if there were biases based on trial design. No differences could be associated with trial design ($P = 0.66$, 0.39 and 0.87 for milk, fat and protein yield, respectively) for the subgroup analysis. Results indicated that dairy cows responded positively to the inclusion of the rumen-protected B-vitamin blend.

Table 1 (Abstr. W71). Effect size for milk, fat and protein yields for lactating dairy cows receiving a rumen protected B vitamin blend

Yield	Effect size	SE	95% CI	*P*-value
Milk, L	0.88	0.14	0.60–1.17	<0.001
Fat, kg	0.04	0.01	0.02–0.05	<0.001
Protein, kg	0.03	0.01	0.02–0.05	<0.001

Key Words: B vitamin, milk yield, milk component

W72 Replacing soybean meal with canola meal reduced enteric methane production and improved milk production in dairy cows. C. Benchaar[1], F. Hassanat*[1], G. Gislon[2], and K. A. Beauchemin[3], [1]*Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre*, *Sherbrooke, QC, Canada*, [2]*Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambiental*, *Milano, Italy*, [3]*Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre*, *Lethbridge, AB, Canada*.

The objective of this study was to determine the effect of replacing soybean meal with canola meal (CM) as protein source in dairy cow diets on intake, milk production, rumen fermentation and enteric CH_4 emissions. Sixteen lactating cows (DIM = 116 ± 23; milk yield = 47.5 ± 4.9 kg) were used in a replicated 4 × 4 Latin square (35-d period; 14-d adaptation). Cows were fed (ad libitum) a TMR composed (on DM basis) of 52% forages (25% grass silage, 22% corn silage; 5% timothy hay) and 48% concentrates. In the concentrate portion, CM was included at 8, 16 and 24% (DM basis) to replace soybean meal and soybean hulls. Diets were formulated to be isonitrogenous at 16.3% CP. Intake, digestibility and milk performance were determined over 6 consecutive days, CH_4 was measured using respiration chambers (5 consecutive days), while rumen fermentation parameters were measured over 2 consecutive days. Linear and quadratic contrasts (Proc MIXED; SAS) were used to determine effects of dietary CM levels on variable responses. Significance was declared at $P \leq 0.05$. Intake (DM) increased linearly with increasing CM inclusion in the diet (26.7, 27.8, 28.0 and 28.6 kg/d for 0 to 24% CM, respectively), while DM and CP digestibility decreased linearly. Canola meal inclusion in the diet increased milk production linearly (44.0, 45.0, 45.6 and 46.2 kg ECM/d for 0 to 24% CM, respectively), but did not affect milk production efficiency. Ruminal pH, total VFA, acetate, an propionate molar proportions averaged 6.15, 141 m*M*, 61% and 23% and were not affected by CM inclusion in the diet. A linear decline in CH_4 emissions (489, 475, 463 and 461 g/d; 5.65, 5.28, 5.05, 4.90% gross energy intake for 0 to 24% CM, respectively) was observed with increasing CM inclusion in the diet. When expressed on milk yield basis, CH_4 emission declined linearly with increasing CM inclusion in the diet (11.5, 11.0, 10.6, 10.3 g CH_4/kg ECM). Results from this study show that replacing soybean meal with canola meal as a protein source in dairy cow diets increases milk production and reduces enteric CH_4 emissions.

Key Words: canola meal, enteric methane, milk production

W73 Canola meal in dairy cow diets: Effect on nitrogen utilization.

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The objective of this study was to determine the effect of replacing soybean meal with canola meal (CM) as protein source in the dairy cow diets on N utilization. Twelve lactating cows (DIM = 116 ± 18; milk yield = 47.1 ± 5.1 kg) were used in a replicated 4 × 4 Latin square (35-d period; 14-d adaptation). Cows were fed (ad libitum) a TMR composed (on DM basis) of 52% forages (25% grass silage, 22% corn silage; 5% timothy hay) and 48% concentrates. In the concentrate portion, CM was included in the diet at 8, 16 and 24% (DM basis) to replace soybean meal and soybean hulls. Diets were formulated to be isonitrogenous at 16.3% CP. Intake of N, N excretion (fecal and urinary), milk N secretion, and MUN were determined over 6 consecutive days; while ruminal NH_3 concentration was determined over 2 consecutive days. Linear and quadratic contrasts (Proc MIXED; SAS) were used to determine effects of dietary CM levels on variable responses. Significance was declared at $P \leq 0.05$. Daily N intake (679, 700, 707 and 718 g/d for 0 to 24% CM, respectively) and N secretion in milk (210, 213, 218 and 222 g/d for 0 to 24% CM, respectively) increased with increasing CM inclusion in the diet. However, efficiency of dietary N use for milk N secretion (N milk/N intake) was not affected by CM inclusion in the diet (averaging 30.8%). Total N excretion (averaged 450 g/d, 64.3 g/100 g N intake) was not affected by CM inclusion in the diet. Fecal N (30.4, 30.9, 31.7 and 32.4 g/100 g N intake) increased linearly with increasing CM inclusion in the diet, whereas a linear decrease in N excretion in urine (35.1, 33.4, 31.7, 31.4 g/100 g N intake) was observed with increasing CM inclusion in the diet. Ruminal NH_3 concentration averaged 6.5 m*M* and was not affected by CM inclusion in the diet, while milk urea N (13.6, 12.7, 12.1, 12.2 mg/dL for 0 to 24% CM, respectively) decreased with increasing CM inclusion in the diet. Results from this study show that replacing soybean meal with canola meal as a protein source in dairy cow diets increases N secretion (g/d) in milk and shifts N excretion from urine to feces, which will reduce potential NH_3 and N_2O emissions.

Key Words: canola meal, nitrogen utilization, dairy cow

W74 Comparison of cow health and performance in 244 European dairy farms before and during OmniGen-AF supplementation.

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Strategies are being implemented in European Union countries to reduce the use of antimicrobials on farm, including alternative husbandry practices and technologies that support animal health and productivity. OmniGen-AF (Phibro Animal Health, Teaneck, NJ) is a complementary feed product composed of yeast, wheat fiber, bentonite and diatomaceous earth that has been shown to influence key markers of immune function in ruminants and other animal models. The objective of this study was to evaluate the practical effects of feeding *OmniGen-AF* on health and milk records in EU dairy farms. Dairy farms from Belgium, France, Italy, the Netherlands, and the United Kingdom (n = 244) were enrolled across all seasons of the year from 2016 to 2019. Farm records were collected for 120 to 180 d before (Pre-OG period) and during feeding 55 g/hd/d of OmniGen-AF to all dry and lactating cows (OG period). Metrics recorded (by number of farms) included: incidence of mastitis (n = 156), retained placenta (n = 150), metritis (n = 131), culled cows (n = 68), milk yield (n = 210) and bulk tank SCC (n = 224). Incidence of mastitis and culled cows was expressed as % total cows over the recording period, while incidence of retained placenta and metritis as % fresh cows. Farm per period was the statistical unit, Pre-OG vs. OG data were analyzed using a paired *t*-test, and statistical significance was declared when $P < 0.05$. Average farm size was 165 cows/farm, and records were collected for average 146 d each period. Overall, the incidence of mastitis, retained placenta (RP) and metritis all decreased ($P = 0.001$) during the OG period, specifically −28% mastitis (18.0% Pre-OG vs. 13.0% OG), −34% RP (14.5% vs. 9.5%), and −34% metritis (18.3% vs. 12.1%); and the number of culled cows decreased by 22% (9.7% vs. 7.5%). Bulk tank SCC decreased an average of 48,000 cells/mL during the OG period (270 vs. 222 × 10^3 cells/mL) while milk yield increased by 0.45 kg/hd/d: 30.6 vs. 31.0 kg/hd/d. These results suggest that feeding OmniGen-AF to dry and lactating cows can have a positive influence on health, milk yield and milk quality on practical field conditions.

W75 Effect of dietary supplementation of polyunsaturated fatty acids on intake, digestibility, milk production, and milk fatty acids of dairy sheep: A meta-analysis.

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We conducted a meta-analysis to examine the effects of dietary supplementation of polyunsaturated fatty acids (PUFA) on intake, digestibility, milk production, and milk fatty acids of lactating dairy. A systematic search in the literature from 1990 to 2019 was conducted and data from 33 peer-reviewed papers with 58 treatments and 1,350 sheep were used to evaluate the magnitude of the effect (effect size) using raw mean differences (RMD) between PUFA supplementation and control. The means were weighted by inverse variance in a mixed model and heterogeneity was calculated by meta-regression and subset analysis using forage (hay, silage, grass), breed (sarda, assaf, crossbreed and others), source (oils, byproducts, mixtures), and level of supplementation as covariates. Dietary levels of PUFA were from 40.1 to 101.5 g/d. Compared with control, PUFA did not affect ($P > 0.05$) DMI (RMD = 0.01 kg/d [−0.01, 0.06]), milk yield (RMD = 0.02 kg/d [−0.06, 0.02]) and milk fat concentration (RMD = −0.08% [−0.22, 0.05]); however, PUFA increased ($P < 0.05$) C18:2 and C18:3 concentrations in milk (RMD = 0.12 g/100 g fatty acid methyl esters (FAME) [−0.1, 0.26], and 0.13 g/100 g FAME [0.06, 0.17]), dry matter digestibility (RMD = 2.2% [1.31, 3.1]), lactose concentration (RMD = 0.08% [0.03, 0.14]), and milk *trans*-10,*cis*-12 CLA (0.03 g/100 g FAME [0.03, 0.04]). In fact, concentrations of total PUFA in milk were increased by 23.9% (RMD = 1.41 g/100 g FAME [1.16, 1.65]), whereas total saturated fat in milk was decreased by 7.6% (RMD = −5.3 g/100 g FAME [−6.6, −3.96]).The subset analysis showed that oils supplementation increased C18:2 in milk (0.31 vs 0.03; −0.12 g/100 FAME; $P < 0.05$), whereas byproducts as a source of PUFA were more effective at increasing C18:3 in milk (0.24 vs 0.12, 0.02 g/100 FAME; $P < 0.05$). Despite the high heterogeneity ($I > 95\%$) observed in this study, PUFA supplementation increased milk quality (increased concentration of unsaturated fatty acids and lactose), but had no effects on the performance of lactating dairy sheep.

Key Words: dairy sheep, fatty acids, meta-analysis

W76 Evaluation of the relationship between ruminal pH and milk de novo fatty acid proportion in early-lactating dairy cows. R. Fukumori*[1], W. Shi[2], S. Oikawa[1], and M. Oba[2], [1]*Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*, [2]*University of Alberta, Edmonton, AB, Canada*.

The objective of this study was to evaluate the relationship between rumen pH and proportion of de novo fatty acids (DNFA) in milk and milk fat, determined by Fourier transform infrared spectroscopy, in lactating dairy cows. Data were collected from 18 multiparous Holstein cows, fitted with a rumen cannula, fed one of experimental diets differing in starch content (22.1 or 28.3%) with or without supplementation of a *Saccharomyces cerevisiae* fermentation product (NutriTek, Diamond V Inc., Cedar Rapids, IA) in a previous study. Milk was sampled on d 7 and 21 after calving, and concentrations of milk fat, de novo fatty acids (DNFA; C6 to C14), mixed origin fatty acids (C16 and C17), and preformed fatty acids ($\geq$C18) were analyzed using CombiScope FTIR A600 (Delta Instruments, Drachten, the Netherlands). Rumen pH was recorded in the ventral sac every 30 s continuously for 72 h, on d 7 to 9 and 21 to 23 after calving, using a pH measurement system (LRCpH; Dascor, Escondido, CA). Pearson correlation coefficients were used to determine the relationship between rumen pH variables and milk fat variables. Milk fat DNFA (g/100 g FA) was positively correlated with nadir ($r = 0.428$, $P < 0.01$) and mean rumen pH ($r = 0.471$, $P < 0.01$), and negatively correlated with duration ($r = -0.511$, $P < 0.01$) and area below rumen pH 5.8 ($r = -0.520$, $P < 0.01$) although milk fat content did not have any relationship with rumen pH variables in this study. The coefficients of determination between rumen pH variables and DNFA were greater in milk fat DNFA (g/100 g FA) than milk DNFA (g/100 g milk), suggesting that milk fat DNFA (g/100 g FA) is an appropriate measurement variable to predict low rumen pH even for cows in early lactation. A regression analysis suggests that cows with subacute rumen acidosis, defined as rumen pH below 5.8 for more than 5 h/d, had milk fat DNFA concentration of 21.9 g/100 g FA or lower. This study showed that milk fat DNFA was correlated with rumen pH variables, and that it has a potential to be used to identify cows with subacute rumen acidosis.

Key Words: de novo fatty acids, rumen pH, subacute ruminal acidosis

W77 Lactational performance in dairy cows receiving a rumen-protected B-vitamin blend replacing unprotected biotin during lactation: Meta-analysis of 13 studies. E. Evans[2], H. Leclerc*[1], and E. Santin[1], [1]*Jefo, St-Hyacinthe, QC, Canada*, [2]*Technical Advisory Services, Bowmanville, ON, Canada*.

This study compared the effects of a rumen-protected blend of B vitamins providing pantothenic acid, pyridoxine, biotin, folic acid and vitamin B$_{12}$ (Jefo, Canada) as a replacement for unprotected biotin on milk, fat and protein yield in cows past peak lactation. Data from 13 on-farm studies (11 double reversal in which the test product was supplied in addition to the currently fed diet in the center period and 2 single crossover design) conducted between 2007 to 2010 were analyzed using Meta-Essentials Software (Creative Commons, 2017). Four of the herds included unprotected biotin in the diet at 10 mg/cow/day, and 9 used unprotected biotin at 20 mg/cow/day. Cows included in results were over 45 d in milk at first milk test, and available for all milk collection periods. All herds participated in registered monthly recording services and all were Holstein herds. Data were available from 2,530 cows (average 194.6 + 147.2 per herd). Effect size was calculated for milk, fat and protein yield based on the treatment differences and standard errors (SE) from the individual trials. Mean effect size, SE and 95% confidence intervals (CI) are given in Table 1. Subgroup analysis was conducted to determine biases based on trial design or level of unprotected biotin (10 vs. 20 mg/cow/day). No differences could be associated with trial design ($P = 0.16$, 0.34 and 0.26 for milk, fat and protein yield, respectively). There were no differences due to level of biotin in the control rations ($P = 0.94$, 0.84 and 0.94 for milk, fat and protein, respectively). Results indicated that dairy cows showed a higher response with the inclusion of the rumen-protected B-vitamin blend in comparison to the dietary supplementation of unprotected biotin.

Table 1 (Abstr. W77). Effect size for milk, fat and protein yields for dairy cows given a rumen-protected B-vitamin blend as a replacement for unprotected biotin

Yield	Effect size	SE	95% CI	*P*-value
Milk, L	0.75	0.29	0.13–1.37	<0.001
Fat, kg	0.05	0.02	0.01–0.09	<0.001
Protein, kg	0.06	0.02	0.01–0.10	<0.001

Key Words: B vitamin, milk yield, biotin

W78 Effects of two levels of starch and monensin on performance of mid-lactation dairy cows. P. Piantoni*, M. A. Messman, B. D. Strang, C. J. Canale, and G. F. Schroeder, *Cargill Animal Nutrition Innovation Center*, *Elk River, MN*.

Sixty Holstein cows (149 ± 17.4 DIM; 37.4 ± 5.7 kg/d milk yield) were used in a randomized block design experiment with a 2 × 2 factorial arrangement of treatments to evaluate the effects of dietary starch (20.1 and 26.7% of diet DM; LS and HS, respectively) and monensin levels (275 and 550 mg/d; LM and HM, respectively) on lactation performance. After a 2-wk covariate period, cows were assigned to 1 of 4 treatments: LSLM, LSHM, HSLM, and HSHM. Treatment diets were fed for 13 wks. Data were analyzed using the GLIMMIX procedure of SAS with fixed effects of starch, monensin, parity, week, and interactions between main effects and parity and week, the random effects of cow and block, and specific covariates. There were no interactions among starch and monensin levels and time for yields of milk, 3.5% FCM, or milk components. However, LSHM decreased DMI compared with the other diets, particularly after 5 wks on treatment (interaction $P < 0.01$). Overall, LS decreased DMI compared with HS (22.9 vs. 24.1 kg/d; $P = 0.01$), and the difference between treatments became larger overtime (interaction $P = 0.09$). LS did not affect 3.5% FCM overall but tended to decrease it toward the end of the treatment period (interaction $P = 0.13$). Therefore, LS increased feed efficiency overall compared with HS (1.57 vs. 1.51; $P = 0.04$), but especially when LS maintained 3.5% FCM yield (interaction $P = 0.03$). Starch level showed a tendency to interact with parity for BW, indicating that HS increased BW in primiparous but not multiparous cows ($P = 0.15$). Compared with LM, HM slightly increased DMI and yields of 3.5% FCM and milk protein and fat in multiparous cows but decreased them in primiparous (all interactions $P < 0.10$). HM decreased milk fat content regardless of starch level (3.34 vs. 3.18%; $P = 0.05$). In summary, results indicate that the interaction between starch and monensin levels affected DMI and feed efficiency, but not yields of milk and milk components, and that lower starch diets increased feed efficiency by decreasing intake and maintaining 3.5% FCM yield. In addition, a higher dose of monensin did not provide additional benefits in mid-lactation dairy cows.

Key Words: energy partitioning, feed efficiency, starch

W79 Feeding frequency effect on behavioral patterns and bacterial community composition in Holstein and Jersey cows. V. M. De La Guardia* and H. A. Paz, *Animal and Dairy Sciences Department, Mississippi State University, $Starkville, MS*.

Multiple factors can influence the composition of the gastrointestinal microbiome of dairy cattle. This study aimed to evaluate the effect of feeding frequency on cow behavior and on the bacterial community composition of the rumen and hindgut. A 28-d trial was conducted with 10 Holstein and 10 Jersey cows fed for ad libitumconsumption a diet comprised of 70% forage and 30% concentrate using the Calan Broadbent Feeding System. Within breed, cows were randomly assigned to treatments: 1 (06:00 h) or 2 (06:00 and 18:00 h) feed deliveries per day. Behavioral patterns were monitored on d 23 and 24 with data recorded at intervals of 15 min and classified into one of the following activities: lying, standing, ruminating, eating, drinking, or milking. On d 28, 5 concomitant collections of rumen and fecal samples were performed at intervals of 6 h via esophageal tubing and fecal grab, respectively. The bacteriome composition of these samples was determined through sequencing of the V4 region of the 16S rRNA gene using the Illumina MiSeq platform. Raw sequences were processed using the QIIME2 pipeline and PERMANOVA was performed using R (v3.5.1). Behavioral data were analyzed using PROC MIXED of SAS (9.4) Behavioral patterns were not affected by feeding frequency within breed ($P \geq 0.15$); however, both ruminating (6.7 vs. 5.5 ± 0.2 h) and laying (15.3 vs. 13.4 ± 0.6 h) times were greater in Holstein than Jersey cows ($P = 0.04$). Shannon diversity was higher for Holstein compared with Jersey cows ($P = 0.01$) and for feces compared with rumen samples ($P < 0.01$). Overall bacterial community composition differed by breed ($P = 0.01$) and sample site ($P < 0.01$) but not by feeding frequency ($P = 0.95$) or time ($P = 0.52$). These results indicate that breed and gastrointestinal site are factors that drive the overall bacterial community composition while feeding frequency and diurnal cycle are not.

Key Words: cattle behavior, feeding frequency, gastrointestinal microbiome

W80 Lactational performance in dairy cows receiving a rumen-protected B-vitamin blend during lactation: Regression analysis to evaluate yield, lactation number and days in milk.

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The objective of this study was to evaluate the impact of a rumen-protected blend of B vitamins pantothenic acid, pyridoxine, biotin, folic acid and vitamin B_{12} (Jefo, Canada) on milk, fat and protein yield in cows and to evaluate the effects of current yield, lactation number and days in milk (DIM). The analysis utilized 5043 observations from 50 on-farm double reversal studies from 7 countries between 2005 and 2015. The test product was added to the current diet in the center period. Cows included were over 45 d in milk at first milk test, and available for all milk collection periods (minimum 28 d). Data were analyzed using a regression model in Minitab 16 (Minitab LLC) with random effects of study, period, days in milk and lactation number. Overall results are provided in Table 1 and an overall description of results is provided in the equation: Milk yield with B vitamin blend = 4.05 + 0.917 × current milk −0.0063 × DIM + 0.246 × lactation number ($R^2 = 0.798$). Current milk yield had a significant effect on response to the B vitamins blend in both primiparous ($R^2 = 0.72$) and multiparous cows ($R^2 = 0.80$). Milk yield and fat yield increased with supplementation, and the extent of change was determined to increase with lactation number ($P < 0.05$). Protein yield increased with the B vitamins blend and was unaffected by lactation number ($P < 0.05$). Responses to the supplementation were found to decline with DIM for milk, fat and protein yield, but to increase for fat and protein percent relative to control ($P < 0.05$). Results indicated that dairy cows responded positively to the inclusion of the rumen protected B vitamin blend with differences in the extent of the response due to lactation number, days in milk and milk yield.

Table 1 (Abstr. W80). Results for all cows from all studies (n = 5,043, DIM =163, Lactation number = 2.20)

Item	Test	Control	*P*-value
Milk, L	33.12	32.22	<0.001
Fat, %	3.72	3.70	0.236
Protein, %	3.23	3.22	<0.551
Fat, kg	1.22	1.17	<0.001
Protein, kg	1.05	1.02	<0.001

Key Words: B vitamins, meta regression, lactational performance

W81 Benefit of zinc methionine hydroxy analog chelate to increasing tissue enrichment with dietary antagonism in Holstein calves.

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Bioavailability of Zn sources is an important component of Zn nutrition of dairy cattle. To expand our knowledge of Zn metabolism, further research is needed to elucidate the impact of antagonisms on Zn enrichment in target tissues. The objective of this trial was to determine the effect of a Zn antagonism, elevated S, on bioavailability of Zn from Zn-glycinate (Zn-Gly) and Zn-methionine hydroxy analog chelate (Zn-MHAC) in Holstein calves. Thirty weaned male Holstein calves (BW = 63.5 ± 2 kg [mean ± SE]) were fed a texturized starter formulated to meet nutrient requirements but varied in sodium sulfate level for 32 d. On d 30, calves were orally administered 0 (n = 6) or 8 (n = 24) mg of Zn from each of 2 sources: ^{67}Zn-Gly and ^{70}Zn-MHAC at 0h. Blood was collected via catheters before isotope administration and 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, and 48 h after isotope administration for determination of isotope enrichment. Calves were euthanized 48 h after isotope administration with target tissues weighed and sampled for determination of isotope enrichment via ICP-MS. Data were analyzed with the MIXED procedure of SAS 9.4, with significance declared at $P < 0.05$ and trends at $0.05 \leq P < 0.10$. Though dry matter intake differed ($P = 0.07$), calves receiving the High diet (11.3 ± 0.2 g S/d) consumed 2.9 times more S than Low diet (3.9 ± 0.2 g S/d). Tissue isotope enrichment decreased ($P < 0.01$) with High S diet for rumen, reticulum, omasum, duodenum, jejunum, ileum, kidney, lung, and spleen ($P = 0.07$). Tissue isotope enrichment increased ($P = 0.08$) with High S diet in thymus. Tissue isotope enrichment was greater ($P \leq 0.05$) with Zn-MHAC compared with Zn-Gly for rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, kidney, lung, heart, spleen, and thymus. No significant interaction of S level and Zn source was observed. The impact of high dietary S on tissue Zn isotope enrichment demonstrates efficacy of this antagonistic model in ruminants. Moreover, changes in Zn enrichment of the splanchnic tissues demonstrates their high metabolic requirement for Zn. Finally, the improved enrichment from Zn-MHAC demonstrates greater bioavailability independent of antagonism presence.

Key Words: bioavailability, stable isotope, zinc

W82 Milk fatty acid profile in dairy cows fed soybean meals and oil versus canola meal and oil.

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This study investigated the effects of feeding diets with solvent-extracted soybean meal (SSBM), canola meal (CM), or extruded soybean meal (ESBM) on an equivalent crude protein (CP) basis on milk fat concentration and yield and milk fatty acid (FA) profile in dairy cows. Fifteen lactating Holstein cows (95 ± 20.0 d in milk and 46.5 ± 7.80 kg/d of milk yield) were used in a replicated 3 × 3 Latin square design experiment with 3, 28-d periods. The diets contained [dry matter (DM)-basis, %]: SSBM, 13.6; ESBM, 14.2; and CM, 17.1; respectively. Soybean or canola oil were added at 0.89 and 0.66% of DM to the SSBM and CM diets, respectively, to achieve similar ether extract content among diets. Milk samples were collected from 4 consecutive milkings during the last wk of each experimental period and analyzed for milk fat concentration and FA profile. Data were analyzed using the MIXED procedure of SAS with treatment and period in the model. Square and cow within square were random effects. Milk fat concentration and yield were not affected ($P \geq 0.66$) by treatment (mean ± SEM: 3.65 ± 0.109%, and 1.56 ± 0.092 kg/d, respectively). Compared with ESBM and SSBM, CM increased ($P \leq 0.03$) proportions of 18:0, 18:1, 20:0, total trans FA, the sum of mono-unsaturated FA and decreased ($P = 0.03$) proportions of de novo synthesized FA in milk fat. ESBM increased ($P \leq 0.001$) the proportions of 6:0, 17:0. 18:2, 18:3, the sum of poly-unsaturated FA and odd and branched-chain FA, compared with both CM and SSBM. The SSBM diet increased ($P \le 0.002$) the proportion of 14:0, 16:0, 16:1, and the sum of saturated FA in milk fat, and decreased ($P < 0.001$) the proportion of preformed FA. In this experiment, inclusion of ESBM, SSBM + soybean oil, or CM + canola oil in the diet did not affect milk fat concentration or yield. The soybean meal diets resulted in greater proportions of 16:0 18:2 and 18:3 while CM had greater proportion of 18:1 in milk fat. Overall, diets modified milk FA profile in a manner expected from the FA concentration of the meals and added oils.

Key Words: soybean meal, canola meal, milk fatty acid

W83 Effects of different levels of aflatoxin B1 on ruminal digestibility, fermentation profile, and N efficiency of a lactating dairy cow diet using a dual-flow continuous culture system. F. X. Amaro*[1], M. Pupo[1,2], B. C. Agostinho[1,3], S. Bennett[1], J. Vinyard[1], L. Tomaz[1,4], R. Lobo[1,5], J. A. Cordeiro[1], Y. Jiang[1], K. G. Arriola[1], A. Faciola[1], A. T. Adesogan[1], and D. Vyas[1], [1]*University of Florida*, *Gainesville, Fl*, [2]*Escola Superior de Agricultura Luiz de Queiroz*, *Piracicaba, SP, Brazil*, [3]*Universidade Estadual de Maringá*, *Maringa, PR, Brazil*, [4]*Universidade Estadual Paulista*, *Botucatu, SP, Brazil*, [5]*Universidade de Sao Paulo*, *Pirassununga, SP, Brazil*.

The objective of this study was to evaluate the effects of increasing doses of aflatoxin B1 (AfB1) on ruminal nutrient digestibility, fermentation profile, and microbial protein synthesis from a total mixed ration (TMR) using a dual-flow continuous culture system. Eight fermentor vessels were used in a replicated 4 × 4 Latin square design with 10-d periods (7 d adaptation and 3 d sample collection). Treatments were Control; no AfB1, AfB50; 50 ppb of AfB1 equivalent to 5.35 μg/d of AfB1, AfB100; 100 ppb of AfB1 equivalent to 10.7 μg/d of AfB1, and AfB150; 150ppb of AfB1 equivalent to 16.05 μg/d of AfB1. Fermentors were fed twice daily (107 g DM/d) with the TMR. The total dose of AfB1 for respective treatments was diluted in ethanol and applied to the fermentors along with feeding. The basal TMR had the same nutrient composition across all treatments (15.9% CP, 26.4% aNDF, 30.3% starch and NE_L of 1.61 Mcal/kg). Rumen fluid was sampled, and pH measured from the vessels at 0, 1, 2, 4, 6 and 8 h after feeding for 2 consecutive days. ^{15}N was used as a marker to assess N efficiency. Data were analyzed using the GLIMMIX procedure of SAS. Linear, quadratic, and control vs. treatment contrasts were tested. Treatments did not affect nutrient digestibility ($P > 0.05$; DM, OM, CP, NDF, ADF), pH or ammonia-N (NH_3-N) concentration. However, sampling time affected pH and NH_3-N concentration ($P < 0.01$) with the lowest pH (5.69) and greatest NH_3-N concentration (20.65 mg/dL) observed at 4 h and 1 h post-feeding, respectively. Total VFA concentration was not affected by treatment; however, sampling time did ($P < 0.01$). Acetate (50.1 vs. 48.9%), propionate (27.5 vs. 26.3%) and acetate-to-propionate ratio (A:P) (1.93 vs. 1.85) were greater for control compared with AfB1 treatments ($P < 0.01$). Bacterial N flow and N efficiency were not affected by treatments. In conclusion, AfB1 addition to the TMR decreased molar proportion of acetate, propionate and A:P ratio without affecting nutrient digestibility or N efficiency.

Key Words: aflatoxin, in vitro, microbial protein

W84 Impact of concentrate allowance on the behavior and production of dairy cows milked in a free-traffic automated milking system. A. J. Schwanke*[1], K. M. Dancy[1], G. B. Penner[2], and T. J. DeVries[1], [1]*Department of Animal Biosciences*, *Guelph, ON, Canada*, [2]*Department of Animal and Poultry Science, University of Saskatchewan*, *Saskatoon, SK, Canada*.

Research to evaluate feeding strategies in automated milking systems (AMS) is ongoing. Concentrated feed is typically used within the AMS to motivate cows to voluntarily visit the milking unit, and the type and amount of concentrate provided may be modified for each cow. The objective of this study was to determine if the allowance of AMS concentrate affects partial mixed ration (PMR) eating behavior, milking activity, and production of dairy cows milked in a free-traffic AMS. Holstein cows (n = 15; 124 ± 53 DIM; parity = 2.7 ± 0.9) were used in a crossover design with 28-d periods including 14 d of adaptation and 14 d of data collection. Cows were housed in a freestall pen with free-traffic access to the AMS. Cows were all provided the same basal PMR; with treatments consisting of a pelleted AMS concentrate allowance of either 3.0 kg/d (L-AMS) or 6.0 kg/d (H-AMS) on a DM basis. Data were summarized by cow and day and analyzed in repeated measures mixed-effect linear regression models. As designed, cows on the H-AMS had greater AMS concentrate provision (5.9 vs. 3.0 kg/d; $P < 0.01$). The standard deviation for mean daily concentrate provision increased from 0.49 to 1.3 kg/d ($P < 0.01$) with greater AMS concentrate allocation. When on the H-AMS, PMR intake was reduced (24.7 vs. 26.3 kg /d; $P = 0.03$) and PMR meal size was smaller (3.2 vs. 3.5 kg /meal; $P = 0.02$). Reduced PMR intake was offset by greater AMS concentrate provision; thus, cows on the H-AMS tended to have greater total DMI (30.6 vs. 29.3 kg/d; $P = 0.06$). On the L-AMS, cows spent +19.3 min/d ruminating ($P < 0.01$), tended to have greater lying bout length (93.7 vs. 85.9 min/bout; $P = 0.07$), and lost body condition (−0.04 vs. +0.13 BCS units; $P = 0.02$). No difference in milk yield was detected between treatments (H-AMS = 46.0 kg/d vs. L-AMS = 44.4 kg/d; SE = 1.09; $P = 0.32$), similarly milking activity (3.9 milkings/d) was not affected by treatment. Overall, these data suggest that allocating a greater amount of concentrate to the AMS, in a free traffic setup, may promote greater total DMI, but may also result in greater day-to-day variation in AMS concentrate provision.

Key Words: robotic milking system, concentrate, feeding management

W85 Potential measurement of daily oxygen consumption and carbon dioxide, methane, and heat production using a spot gas sampling technique in cattle. C. Lee*[1], K. A. Beauchemin[2], K. Nichols[3], D. L. Morris[4], J. Dijkstra[3], P. J. Kononoff[4], and D. Vyas[5], [1]*Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University*, *Wooster, OH*, [2]*Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada*, *Lethbridge, AB, Canada*, [3]*Animal Nutrition Group, Wageningen University and Research*, *Wageningen, the Netherlands*, [4]*Department of Animal Science, University of Nebraska-Lincoln*, *Lincoln, NE*, [5]*Department of Animal Sciences, University of Florida*, *Gainesville, FL*.

Spot measurement from breaths for CO_2 and CH_4 (e.g., Greefeed system) has been used to estimate daily CO_2 and CH_4 production. This study was conducted to examine accuracy of daily O_2 consumption and CO_2, CH_4, and heat production estimated using a spot sampling technique. Data from 3 studies where daily O_2 consumption and CO_2, CH_4, and heat production measured using respiratory chambers were used: Study 1, 8 beef heifers (gases measured every 30 min over 3 d); Study 2, 56 Holstein lactating cows (gases measured every 12 min over 3 d); Study 3, 12 Jersey lactating cows (headbox chamber; gases measured every hour for 1 d). Within study, averages of all measurements (ALL) and averages of measurements from 12, 8, 6, and 4 spot samples (i.e., every 2, 3, 4, and 6-h sampling in a 24 h cycle, respectively; FQ12, FQ8, FQ6, and FQ4, respectively) were compared. Within study, PROC MIXED was used to compare variables between ALL and each frequency and examined an interaction of diet by frequency. PROC REG was used to evaluate accuracy of spot sampling within study [i.e., ALL (observed) vs. FQ12, FQ8, FQ6, or FQ4 (predicted)]. No frequency effect was observed for variables except that CO_2 was lower (5,411 vs. 5,552 L/d; $P < 0.01$) for FQ4 compared with ALL and FQ12, FQ8, and FQ6 in Study 2. No interaction of diet by frequency was observed for all variables except that CH_4 tended to have an interaction in Study 1 ($P = 0.08$), i.e., a diet effect ($P < 0.01$) with ALL, FQ12, and FQ8 but no effect with FQ6 and FQ4. A regression analysis between ALL and FQ showed that intercepts were different from 0 ($P < 0.01$) and slopes were different from 1 ($P < 0.01$) for almost all variables. However, most variables for FQ12 and FQ8 had root mean square prediction errors lower than 8% of the mean and concordance correlation coefficients greater than 0.80. In conclusion, spot gas sampling from chambers using 12 or 8 sampling frequencies (2- or 3-h intervals in a 24 h cycle) has potential to accurately estimate daily O_2 consumption and CO_2, CH_4, and heat production in cattle.

Key Words: gaseous exchange, heat production, spot gas sampling

W86 Responses in milk performance and feed intake of transition dairy cows supplemented with coated choline chloride. L. S. Royo*[1], M. Puyalto[1], J. J. Mallo[1], G. Elocoso[2], J. Ragues[2], and A. Bach[3,4], [1]*Norel SA*, *Madrid, Spain*, [2]*Blanca from the Pyrenees*, *Hostalets de Tost, Spain*, [3]*ICREA, Institució Catalana de Recerca i Estudis Avançats*, *Barcelona, Spain*, [4]*Department of Ruminant Production, IRTA*, *Caldes de Montbui, Spain*.

The aim of this study was to evaluate the effect of dietary supplementation of coated choline chloride (Chol) on the performance of dairy cows in the transition period. Sixty Holstein cows (606 ± 84 kg BW; 21 primiparous (PMC) and 39 multiparous (MPC) cows) were randomly distributed in 2 groups (n = 30) and exposed for 60 d to 2 treatments following a complete randomized design. Treatments consisted of supplementation with 60 g/d of Chol (CLN) 3 wk before and after calving, or no supplementation in the group control (CTR). Cows were fed a TMR during the dry period (12.2% CP, 57.3% NDF, 1.30 Mcal of NEl/kg; DM basis) and a TMR during the fresh period (15.3% CP, 32.2% NDF, 1.69 Mcal of NEl/kg; DM basis) twice daily. On a daily basis, DMI, milk yield, and milk fat and protein content were determined individually. Animal was the experimental unit and data were analyzed using a mixed-effects model for repeated measures. Feed intake and milk yield were affected by an interaction ($P < 0.01$) among treatment, week, and parity, which resulted in lower DMI but high milk yield in MPC on CLN compared with MPC on CTR during some days of early lactation. Milk fat content (%) and yield (kg/d) were affected by an interaction ($P < 0.01$) among treatment, week, and parity, with MPC on CLN having greater milk fat content and yield than MPC on CTR during a few days of early lactation. Milk protein yield (kg/d) was affected by an interaction ($P < 0.01$) among treatment, week and parity, with MPC on CLN having greater milk protein yield the last week of the trial. As result, ECM was affected by an interaction among treatment, week, and parity. It is concluded that Chol supplementation during the transition period has potential to improve milk yield, milk fat content, milk fat yield, and ECM, but also decreases DMI during some days during early lactation in MPC, but not PMC, without affecting feed efficiency.

Key Words: milk production, rumen protected, transition

W87 Evaluation of high-quality alfalfa silage in a high-forage diet for lactating dairy cows. C. M. Engel*[1], D. K. Combs[1], and K. F. Kalscheur[2], [1]*University of Wisconsin, Madison*, *Madison, WI*, [2]*US Dairy Forage Research Center, USDA-ARS*, *Madison, WI*.

High quality forages improve nutrient digestibility, reduce gut fill, and allow greater intake of protein and forage NDF in dairy cow diets. An experiment was conducted to determine how much high-quality alfalfa silage (AS) could be utilized in a high-producing dairy cow diet to replace concentrate feedstuffs without lowering milk production. Forty-eight lactating Holstein cows (24 multiparous, 24 primiparous, 141 ± 22 DIM) were randomly assigned to 4 treatments in a randomized complete block design. The experiment had a 2-wk covariate period followed by an 8-wk treatment period. Diets consisted of 40% BMR corn silage, 10% conventional AS and either 0, 6, 12, or 18% AS (0AS, 6AS, 12AS, 18AS, respectively) on a DM basis. Diets were formulated to contain about 30% NDF, 26.4% starch, and 17.4% CP on a DM basis. Animals were housed in a tie-stall barn, milked 3 times a day and fed once daily. Data were analyzed using PROC MIXED of SAS. The model included covariate, treatment, parity, and week as the repeated measure. Increasing high quality AS in the diets linearly decreased DMI ($P < 0.05$). Because milk production was unaffected ($P > 0.10$) by diet, feed efficiency (FE = energy-corrected milk/DMI) increased linearly from 1.63 to 1.83 when high quality AS incrementally increased in the diets (Table 1). Milk fat % and yield increased linearly as AS replaced concentrate feedstuffs. Percent and yield of both milk protein and lactose did not differ among the treatments. Milk fat % and yield, lactose %, and FE were greater for primiparous cows than multiparous cows. Substitution of protein and non-forage fiber feedstuffs up to 18% of the diet (DM basis) with high quality AS did not reduce milk production and increased milk fat yield, milk fat % and FE.

Table 1 (Abstr. W87).

	Treatment					
Item	0AS	6AS	12AS	18AS	SEM	P-value[1]
DMI, kg/d	26.3	26.5	25.4	24.9	0.37	L
Milk, kg/d	43.7	43.0	43.3	42.6	0.74	NS
Fat, %	3.35	3.76	3.72	3.90	0.07	L, C
Protein, %	3.02	3.03	3.01	2.99	0.02	NS
Lactose, %	5.08	5.08	5.07	5.09	0.01	NS
FE	1.63	1.69	1.75	1.83	0.03	L

[1] L = linear effect ($P < 0.05$); Q = quadratic effect ($P < 0.05$); C = cubic effect ($P < 0.05$); NS = not significant ($P > 0.05$).

Key Words: high-quality alfalfa, forage, milk

W88 Effects of ground flaxseed on ruminal microbiome composition in Jersey cows during the grazing season. D. C. Reyes*[1], E. A. Latham[2], L. H. P. Silva[1], B. J. Isenberg[1], and A. F. Brito[1], [1]*University of New Hampshire*, *Durham, NH*, [2]*Bezoar Laboratories*, *Bryan, TX*.

Ground flaxseed (GFLX) has been used as a supplement to increase the energy density of dairy diets and the concentration of polyunsaturated fatty acids in milk fat, which have multiple benefits for human health. However, the impact of GFLX on the ruminal microbiota profile of grazing dairy cows remains poorly understood. Therefore, the effects of supplementing cool-season herbage with 10% of diet DM as GFLX on the ruminal microbiota composition of organic certified Jersey were examined in the present study. Ten lactating Jersey cows (112 ± 49 DIM) were blocked in pairs by milk production or DIM and, within pair, randomly assigned to control (CON; soybean meal and ground corn grain mix as 10% of total DMI) or GFLX (10% of total DMI) in a randomized complete block design experiment with repeated measures over time. Each experimental period lasted 28 d (n = 4). Ruminal fluid samples were collected after the morning milking (~0700 h) for 2 consecutive days using a stomach tube. Microbial diversity of ruminal samples was determined using the 16s rRNA gene (V1-V3 region) and the Illumina MiSeq sequencing platform. Sequencing data were processed and analyzed with QIIME software. The statistical model included the fixed effects of treatment, period, block, their interactions, and the random effects of cow nested in treatment and was run in SAS with repeated measures. The most abundant phyla (*Firmicutes* and *Bacteroidetes*; 49.2 and 40.7% relative abundance (RA), respectively; $P > 0.05$), families (*Sphingobacteriaceae* and *Clostridiacea*; 11.9 and 10.0% RA, respectively; $P > 0.05$), and genera (*Mucilaginibacter* and *Rikenella*; 9.3 and 8.8% RA, respectively; $P > 0.05$) were unaffected by GFLX. However, GFLX increased ($P < 0.05$) the RA (%) of the bacterial phylum *Lentisphaerae* (0.002 vs. 0.0007 ± 0.001) and tended ($P = 0.08$) to increase those of *Tenericutes* (2.22 vs. 1.47 ± 0.22) compared with the CON. At a 10% DMI inclusion level, GFLX did not impact rumen microbiota of grazing Jersey cows.

Key Words: organic, microbiome, pasture

W89 Investigating the effectiveness of macroalgae species on methane production and rumen fermentation in vitro. D. E. Wasson*[1], H. Stefenoni[1], S. Welchez[1], C. Lage[1], S. Räisänen[1], A. Melgar[1], M. Fetter[1], C. Yarish[2], and A. N. Hristov[1], [1]*The Pennsylvania Univer-*

sity, *University Park, PA*, [2]*The University of Connecticut*, *Storrs, CT*.

Due to implications for global climate changes, methane (CH_4) emissions coming from livestock industries have been the topic of ongoing research. This experiment investigated the effect of macroalgae on methanogenesis and rumen fermentation in vitro. Twenty species of macroalgae were analyzed for their effect on gas production and composition and volatile fatty acid (VFA) concentrations in vitro. Incubations were duplicated and carried out for 24 h with rumen inoculum taken from 2 cannulated lactating Holstein cows fed a standard corn silage/alfalfa haylage and concentrate feeds diet. Treatments were tested in triplicate, at an inclusion rates of 0.5, 1.0, and 2.0% of feed dry matter (DM). Gas production was continuously monitored using an automated gas production system and gas samples were collected at 12 h and 24 h for analysis of CH_4 and hydrogen (H_2) concentrations. VFA concentrations were analyzed at incubation endpoint. Data were analyzed by incubation set with the MIXED procedure of SAS with treatmnent in the model. When compared with CON (basal total mixed ration), 24 h gas production was increased ($P \leq 0.05$) 5.8 to 7.2% by *Fucus vesiculosus*, *Mastocarpus papillatus*, and *Sargassum fluitans*. *Asparagopsis taxiformis* (AT) decreased ($P = 0.001$) 24 h gas production by 17.4%, when compared with CON. At 24 h, CH_4 was decreased ($P < 0.001$) 98% by AT, compared with CON. Compared with CON, H_2 production at 24 h was increased by AT ($P < 0.001$; from not detectable to 2.19 mL/g DM). There was no effect of treatment on total VFA concentrations. AT decreased ($P < 0.001$) molar proportion of acetate by 6.3%. Molar proportion of proprionate was increased ($P = 0.02$) by AT 9.6%, compared with CON, and acetate:proprionate ratio was decreased ($P = 0.01$) by 13.6%, suggesting a shift in VFA profile likely due to increased H_2 concentration. Overall, with the exception of *Asparagopsis taxiformis*, no other macroalgae decreased enteric methane production in this in vitro experiment.

Key Words: enteric methane, macroalgae, rumen fermentation

W90 Effects of increasing biocholine levels on productive performance of dairy cows. A. Nunes[1], L. Ghizzi[1], G. Silva[1], M. Dias[1], T. Silva[1], N. Grigoletto[1], R. Chesini[1], P. Vittorazi Junior[1], P. Curti[1], M. Syperreck[2], and F. Rennó*[1], [1]*University of São Paulo*, *Pirassununga, SP, Brazil*, [2]*Nutriquest Tecnofeed Animal Nutrition Ltda*, *Campinas, SP, Brazil*.

The aim of this study was to evaluate the effects of increasing biocholine levels (Biocholine powder, Techno Feeds Brasil Ltda) on dry matter intake, milk yield and composition, productive efficiency and sorting index of dairy cows. Twenty-four Holstein cows [28.6 ± 4.4 (mean ± SD) kg milk yield per day, 160 ± 67 d in milk, 636 ± 83.2 kg BW] were enrolled in a 4 × 4 Latin square experimental design with 21 d periods and sampling performed during the last 7 d. Treatments consisted of a control (basal diet with no supplementation), and diets with 7, 14 and 21 g/cow/d of biocholine. Individual dry matter intake and milk production were recorded daily. Cows were milked twice daily and milk samples were collected for 3 consecutive days. Total mixed ration and orts were sampled for 2 d to evaluate sorting index, which was calculated as effected intake corresponding to each sieve of the particle separator (Penn State Particle Separator) expressed by percentage of total predicted intake. Data were analyzed using the MIXED procedure of SAS 9.4, using linear and quadratic contrasts to evaluate dose response of biocholine inclusion and control vs. biocholine contrast. Biocholine inclusion quadratically affected ($P = 0.031$) milk yield, tended to have a quadratic effect ($P = 0.052$) on protein and lactose ($P = 0.056$) yield, with maximal estimated yields at 7 and 14 g/cow/d, respectively. Furthermore, there was a tendency ($P = 0.082$) for a quadratic effect on milk production efficiency, with the highest estimated value at 7 g/cow/d. Biocholine quadratically increased selection of particles greater than 19 mm ($P = 0.010$) and decreased selection of particles between 8 and 19 mm ($P = 0.003$). There were no effects on feed intake, fat-corrected milk, milk fat yield, and milk fat, protein and lactose content. Therefore, biocholine inclusion increases dairy cow performance.

Key Words: additive, milk yield, vitamin

W91 Productive performance of dairy cows feed with soybean silage and oat silage. T. Silva, L. Ghizzi, G. Silva, A. Nunes, M. Dias, N. Grigoletto, L. Gheller, J. Marques, T. Del Valle, L. Sakamoto, L. Fernandes, and F. Rennó*, *University of São Paulo*, *Pirassununga, SP, Brazil*.

Use of corn silage for dairy cows is very common, but alternative silages, such as oat and whole soybean can contribute as protein and energy sources to these animals. The objective of the present study was to evaluate the partial replacement of corn silage supplied to dairy cows by soybean silage or black oat silage and its effects on intake and apparent total digestibility of dry matter and nutrients, milk yield and composition, feed behavior and sorting index. Twenty-four lactating Holstein cows were used, distributed in a 3 × 3 Latin square experimental design, receiving the following treatments: 1) corn silage (CS), using corn silage as exclusive forage; 2) soybean silage (SS), using 16% substitution of corn silage for whole soybean silage; and 3) Oat silage (OS), with 16% substitution of corn silage by black oat silage. Data were analyzed using the MIXED procedure of SAS 9.4, and differences between treatments were estimated by the Tukey test, with significancy level of 5%. Inclusion of OS and SS decreased dry matter intake, organic matter and crude protein compared with CS ($P \leq$ 0,009). Diet with SS decreased neutral detergent fiber (NDF) intake ($P \leq$ 0,004), while diet OS decreased ether extract intake ($P <$ 0,001). Apparent total-tract digestibility of dry matter and nutrients was not influenced by the inclusion SS or OS ($P >$ 0,069). OS decreased milk yield and milk protein yield compared with SS and CS ($P <$ 0,001), however inclusion of SS and OS did not influence fat-corrected milk, milk fat yield, milk fat, protein and lactose contents and fat-corrected milk efficiency ($P \geq$ 0,062). OS increased selection of particles less than 4 mm, while SS presented an intermediate value ($P =$ 0,049). Diets with SS and OS increased ruminating and chewing activity ($P \leq$ 0,029), but only SS increased ruminating and chewing activity (min/kg NDF intake) compared with control diet, while OS presented intermediate value ($P \leq$ 0,004). Replacing CS for OS negatively influenced dry matter intake and milk yield of dairy cows. In contrast, replacing CS with SS negatively influenced dry matter intake, however milk yield was the same as CS, can be an alternative source of forage.

Key Words: silage, productive performance, milk yield

W92 Natural additives increase production performance of dairy cows during the summer. N. Grigoletto[1], M. Dias[1], G. Silva[1], L. Ghizzi[1], A. Nunes[1], T. Silva[1], R. Chesini[1], P. Vittorazi Junior[1], J. Pettigrew[2], L. Costa e Silva[3], A. Koontz[3], and F. Rennó*[1], [1]*University of São Paulo*, *Pirassununga, SP, Brazil*, [2]*James Pettigrew Consulting*, *Nicholasville, KY*, [3]*Alltech Inc*, *Nicholasville, KY*.

The objective of this study was to evaluate the effects of Milk Sacc (a proprietary blend of live yeast culture, prebiotics, and organic minerals, Alltech Inc., Nicholasville, KY, USA) and monensin on feed intake, milk yield and composition, productive efficiency, sorting index, feed behavior, and thermal status of dairy cows during the summer (temperature means 24°C ± 1°C, relative humidity 73% ± 3% and temperature-humidity index [THI] 73 ± 1). Forty lactating Holstein cows [27.2 ± 5.25 kg/d milk yield, 187 ± 115.4 DIM, 637 ± 97.5 kg BW] were blocked by parity, DIM and milk yield. Cows within each block (n = 10) were randomly distributed to one of the following treatments: control (CO; basal diet without feed additives), monensin (MO; basal diet with 20 mg of monensin/kg DM), Milk Sacc (MS; basal diet with 40 g of Milk Sacc/cow/d), and Monensin + Milk Sacc (MM; MO and MS combined). Feed additives were added to each concentrate. The experiment lasted 11 weeks, with the 2 initial weeks used as covariate and the following 9 weeks used for sampling and data collection. Individual DMI and milk yield were recorded daily. Sorting index (the Penn State Particle Separator is characterized by 4 screens: P1 > 19 mm; P2 = 19 to 8 mm; P3 = 8 to 4 mm; P4 = closed bottom < 4 mm), feed behavior, and thermal status were evaluated every 3 weeks during 2 consecutive days. Data were analyzed using the MIXED procedure of SAS 9.4. Milk Sacc increased ($P = 0.036$) milk yield, ($P =$

0,028) fat-corrected milk, ($P = 0,023$) milk fat, ($P = 0,010$) protein, and ($P = 0,010$) lactose yields. Moreover, Milk Sacc increased DMI expressed as a percentage of BW ($P = 0.022$). Milk Sacc increased ($P = 0.021$) sorting index for particles between 8 and 19 mm and decreased ($P = 0.040$) for particles smaller than 4 mm when compared with control. Milk Sacc decreased ($P = 0.030$) time of rumination (min/d) and ($P = 0,035$) chewing (min/kg NDF). However, MS had no effects on thermal status. Monensin did not differ to the CO. Milk Sacc may be a useful option during the summer due its ability to increase milk yield, and milk component yields.

Key Words: heat stress, Milk Sacc, milk composition

W93 Effects of the addition of organic acids on TMR and feeding frequency on productive performance of dairy cows.

M. Dias[1], G. Silva[1], L. Ghizzi[1], N. Grigoletto[1], T. Silva[1], A. Nunes[1], R. Chesini[1], P. Vittorazi Junior[1], J. Pettigrew[2], A. Koontz[3], L. Costa e Silva[3], and F. Rennó*[1], [1]*University of São Paulo*, *Pirassununga, SP, Brazil*, [2]*James Pettigrew Consulting*, *Nicholasville, KY*, [3]*Alltech Inc*, *Nicholasville, KY*.

The aim of this study was to determine the effects of organic acids (OA; Mold-Zap, Alltech Inc., Nicholasville, KY) on total mixed ration (TMR) temperature and the frequency of TMR supply on intake and total apparent nutrient digestibility, milk yield and composition, sorting index and feeding behavior of dairy cows. Twenty-four Holstein cows were used in a 4 × 4 Latin square experimental design and randomly assigned to the following treatments: Control-1, basal diet, offered once a day, in the morning; OA-1, basal diet associated with OA (500 mL/1000 kg natural matter), offered once a day, in the morning; Control-2, basal diet, offered twice a day, in the morning and early afternoon; and OA-2, basal diet associated with OA (500 mL/1000 kg natural matter), offered twice a day, in the morning and early afternoon. TMR was placed in artificial troughs simulating the feeding where OA were added and temperature was recorded every 30 min for 24 h. Each experimental period lasted 21 d, being 14 d for acclimation and 7 d for data collection. Individual dry matter intake and milk yield were recorded daily and milk samples were collected for 3 consecutive days, during the morning and afternoon milking. Feed behavior was evaluated for 2 consecutive days every 5 min. Feedstuffs, orts, and feces were analyzed for the contents of DM, crude protein, ether extract, ash, NDF, and starch. Indigestible NDF was used as internal marker to estimate daily fecal DM excretion. Data were analyzed using the MIXED procedure of SAS (2001). There was an increase in milk and protein yield and milk lactose content ($P < 0.005$) with the presence of OA on TMR. The use of OA resulted in a tendency to increase fat-corrected milk yield ($P = 0.078$). However, OA had no influence on intake, nutrient digestibility, sorting index, and milk total solids content ($P > 0.005$). Cows fed twice a day selected more particles smaller than 4 mm ($P = 0.012$). The use of OA decreased the time (min/d) remained standing ($P = 0.019$) and idling ($P = 0.045$). Therefore, the inclusion of OA on TMR of dairy cows resulted in better production performance.

Key Words: additive, performance, feed behavior

W94 Effects of a flavonoid-rich extract on plasma sirtuin 1 and metabolic biomarkers in mid-lactation Holstein cows.

S. T. Quanz*[1], L. K. Mamedova[1,2], D. E. Schimek[3], S. E. Schuling[3], and B. J. Bradford[1,2], [1]*Kansas State University*, *Manhattan, KS*, [2]*Michigan State University*, *East Lansing, MI*, [3]*NutriQuest*, *Mason City, IA*.

Sirtuins have recently been shown to influence the antioxidant regulation of several cellular functions. Sirtuin1 (Sirt1), an intracellular deacetylase enzyme, is central in protecting the cell from reactive oxygen species. The aim of this study was to assess the effect of a commercially available flavonoid-rich extract (FRE) in mid-lactation cows. Mid-lactation Holstein cows (n = 31) producing ≥40.8 kg milk/d were blocked by parity, days in milk, and milk production, then randomly assigned to treatment within block. Treatment cows received 10 g/d of FRE (Relieve, NutriQuest, Mason City, IA) administered in a pellet distributed by an automated milking system at each milking, whereas control received a nutritionally equivalent pellet without the FRE. Cows were housed in a freestall barn equipped with a robotic milking system which they entered free-choice throughout the day. Blood plasma was collected from the coccygeal vein before treatment and on d 30 and 60 of the study. Statistical models evaluated fixed effects of covariate data, treatment, parity group (1, 2, or 3+), block, and time, with cow as a random effect; significance was declared at $P < 0.05$. There were no overall treatment effects on plasma concentrations of free fatty acids (FFA), advanced oxidation protein products (AOPP), Sirt1, trolox equivalent antioxidant capacity (TEAC) or β-hydroxybutyrate (BHB; all $P > 0.10$). Across treatments, Sirt1, TEAC, and FFA increased over time (d 60 vs. d 30) while AOPP decreased. Parity 3+ cows had greater Sirt1 concentrations than parity 1 and tended ($P = 0.07$) to be greater than parity 2. There were parity × time interactions for Sirt1 and FFA. Parity 1 cows had a greater increase in Sirt1 over time compared with parity 2 cows. Likewise, FFA concentrations increased to a greater extent for parity 2 cows vs. parity 1 and parity 3+ cohorts. No significant associations were found between concentrations of Sirt1 and other measured biomarkers in plasma. In conclusion, FRE supplementation did not alter plasma metabolite concentrations in mid-lactation cows that were likely not under oxidative stress.

Key Words: metabolites, sirtuin, oxidative stress

W95 The effect of fiber properties on intake in a sub-tropical partial mixed ration dairy system.

K. A. D. Ison*[1,2], M. A. Benvenutti[1], D. G. Mayer[1], and D. G. Barber[1], [1]*Queensland Department of Agriculture and Fisheries*, *Gatton, QLD, Australia*, [2]*The University of Queensland*, *Gatton, QLD, Australia*.

Partial mixed ration (PMR) dairy systems incorporate both a mixed ration and pasture in the diet of milking cows. This system has recently increased in popularity throughout the sub-tropical regions of Australia due to more irregular seasons leading to reduced pasture growth. However, the ideal strategies to maximize total intake, and the interactions between the 2 distinct diet components are largely unknown within sub-tropical PMR systems. The aim of this experiment was to identify how variability in fiber properties; 24h neutral detergent fiber (NDF) digestibility (24h NDFD), indigestible NDF (iNDF) and mincing energy (ME; indicates the physical resistance to chewing and rumination) within the mixed ration (MR) effect intake. The study took place at the Gatton Research Dairy, Queensland, Australia, during spring 2019. The experiment treatments were 3 diets composed of either a maize, white sorghum or forage sorghum silage base, with varying concentrate quantities to achieve differences in fiber properties while balancing for NDF (Table 1). All cows were offered 12.5 and 10 kg dry matter (DM) of MR and lucerne pasture. Diet 3 had the lowest 24h NDFD and highest iNDF (Table 1), however tended to have higher pasture and total intakes, 1.0 (SEM = 0.62, $P = 0.40$) and 2.0 (SEM = 0.17, $P = 0.17$) kg DM respectively more than Diet 1 or 2. Diet 3 was lowest in ME, indicating less chewing and ruminating was required to allow particles through the rumen, likely leading to increased passage rate and consequently higher intakes. Although not significant, these results indicate that estimating accurate intakes within PMR systems from chemical fiber characteristics alone, is likely inadequate. Future work should investigate the physical attributes of fiber such as mincing energy, which coupled with chemically measured fiber properties may improve the accuracy of intake estimates.

Table 1 (Abstr. W95). Chemical and physical fiber characteristics of each treatment

Item	Diet 1	Diet 2	Diet 3
NDF (%DM)	27.9	27.6	27.7
24h NDFD (%DM)	40.9	37.4	33.2
iNDF (%DM)	9.8	10.7	11.0
Mincing energy (J/g DM)	35.4	26.4	23.8

Key Words: partial mixed ration, fiber digestibilty

W96 Effects of dietary antioxidants and modulators of immune response on milk fatty acids as determined by mid-infrared spectroscopy in Holstein cows during heat stress.

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Mid-infrared spectroscopy can be used to obtain predictions of milk fatty acid (FA) profiles and thus serve to identify risk of heat stress in cows. Twelve multiparous Holstein cows (42.2 ± 5.6 kg milk/d; 83.4 ± 27.1 DIM) were used in a split-plot design testing the effects of mineral and vitamin supplementation on milk FA during heat stress. The main plot was the level of dietary vitamin E and Se: 1) HVE: 200 IU/kg and 1.2 ppm; LVE: 20 IU/kg and 0.3 ppm; respectively). Within each plot, cows were randomly assigned to 1) heat stress (HS), 2) Pair-feeding (PF), or 3) HS with Vitamin D and Ca supplementation (HS+DCa; 1820 IU/kg and 1.5% Ca) in a Latin square design with 14-d periods. Milk FA were analyzed by mid-infrared spectroscopy in milk samples from d 0, 3, 7, 10, and 14. This statistical model included the effect of cow and period as random effects, and the fixed effects of plot, treatment, day and their interactions. A main plot x time interaction was observed for milk fat concentration of FA <16C (de novo origin; $P = 0.08$) with a progressive decrease over time in both groups, but lower concentrations on d 3, 7, and 14 in the LVE than in the HVE group (−7%; $P < 0.05$). A 3-way interaction was detected for the concentration of 16 C FA in milk fat (mixed origin; $P = 0.03$), as they decreased in the PF treatment, being lower than in HS and HS+DCa on d 7, 10, and 14 (−14%) in the HVE, and on d 7 and 14 on the LVE group (−13.5%; $P < 0.05$). A 3-way interaction was detected for FA >16C ($P = 0.02$; preformed FA) as their concentration in milk fat was higher in PF than in HS and HS+DCa cows on d 7, 10, and 14 in the HVE group (+21%; $P < 0.05$), but not different at any time point in the LVE group. Hence, mineral and vitamin supplementation modulates the effects of heat stress on milk fatty acid profile. Milk FA profile by mid-infrared spectroscopy could serve as an indicator of heat stress risk in dairy farms.

Key Words: mid-infrared spectroscopy, heat stress, milk fatty acids

W97 Evaluation of brown midrib sorghum silage in the diets of lactating dairy cows.

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Brown midrib (BMR) sorghum is an alternative forage that can potentially replacement corn silage (CS). The objective of this study was to evaluate the replacement of BMR CS with increasing concentrations of BMR sorghum silage (SS) while maintaining or not maintaining starch concentration in the diets of high producing dairy cows. Sixty Holstein cows in mid-lactation (162 ± 41 DIM) were assigned to treatments in a randomized complete block design. Cows were fed a common covariate diet for 2 weeks followed by experimental diets for 8 weeks. Diets were formulated to contain 39% BMR corn silage, 19% alfalfa silage, and 42% concentrate (DM basis). Sorghum silage was included in diets at 0, 10, or 20% of the diet. Because SS had very little starch, diets with SS were formulated to either decrease in diet starch concentration or additional high moisture corn (+C) was included replacing soybean hulls to maintain starch concentration compared with 0SS (25% starch). Data were analyzed using MIXED procedures of SAS. Orthogonal contrasts determined the effect of adding BMR SS, corn grain, or their interaction. Dry matter intake (DMI) decreased as SS replaced CS ($P < 0.05$; Table 1). Similarly, milk production decreased from 39.4 kg/d for cows fed 0% SS to 36.8 kg/d for cows fed 20% SS. Milk fat % increased when SS was included, but protein and lactose % was not affected. Milk protein yield decreased ($P < 0.05$) with the inclusion of SS, but milk fat yield and energy-corrected milk (ECM) was not affected by diet. Feed efficiency (ECM/DMI) increased as SS was included because DMI decreased as ECM remained similar for all diets. The addition of +C to match dietary starch concentration with the 0SS diet did not improve milk production within each inclusion level of SS.

Table 1 (Abstr. W97).

	BMR sorghum silage, %						
Item	0SS	10SS	10SS+C	20SS	20SS+C	SEM	P^1
DMI, kg/d	25.8	24.3	23.9	22.8	23.0	0.61	F, L
Milk, kg/d	39.4	37.9	38.6	36.9	36.7	0.64	F, L
Fat, %	3.47	3.97	3.78	3.97	3.88	0.10	F
Protein, %	2.96	2.99	3.01	2.93	2.90	0.05	NS
ECM, kg/d	39.1	39.6	39.3	39.2	38.0	1.00	NS
FE	1.53	1.63	1.64	1.74	1.66	0.06	F

[1]F = forage type: no SS vs. SS ($P < 0.05$); L = level of SS inclusion ($P < 0.05$); NS = not significant ($P > 0.05$).

Key Words: brown midrib (BMR) sorghum silage, forage, dairy cow

W98 Effect of dietary supplementation with live yeast on lactation performance and milk fatty acid profile in high-producing dairy cows.

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Objectives were to evaluate the effects of dietary supplementation with live yeast (2.10^{10} cfu/g *Saccharomyces cerevisiae;* ActiSaf Sc48) on milk yield and composition, DMI, body condition score (BCS), BW, and milk fatty acid (MFA) profile of high-producing dairy cows. Multiparous (n = 96) and primiparous (n = 32) cows were stratified by parity, DIM, and milk production, and randomly assigned to 16 pens of 8 cows each in a freestall barn. Cows were milked 2X daily, and pen-fed a basal diet containing 16.3% CP, 1.56 NE_L/kg DM, 26.9% aNDFom, and 28.8% starch. The experiment was a complete randomized design with a 2-week covariate adjustment, followed by a 12-week treatment period. Pens were randomly assigned to 1 of 2 treatments: 1) Control (CO), basal diet + 354 g/cow/d of dried distillers grains (DDG), and 2) Live Yeast (YE): Basal diet + 349 g of DDG and 5 g/cow/d of live yeast. Mixes were incorporated into the TMR. Individual milk yields were recorded daily and milk composition determined weekly. Pen DMI was recorded daily. BCS and BW were determined during the covariate period and then wk 4, 8 and 12 of the treatment period. Composited milk samples by pen were analyzed for milk fatty acid profile by GC analysis. Data were analyzed with a linear model with repeated measurements using PROC Mixed of SAS, with pen as the experimental unit. The YE treatment decreased ($P = 0.03$) somatic cell count (SCC; 88.8 vs 61.4 cells/mL [back-transformed data], respectively CO and YE), with decreases observed at wk 1–4, 10 and 12 of experimental period (treatment by week interaction: $P = 0.09$), but no changes in other milk components, milk yield (49.7 vs 49.1 kg/d; $P = 0.13$), or DMI (28.6 vs 28.3; $P = 0.57$). BW and BCS were unaffected by treatment. In addition, the YE treatment tended ($P = 0.07$) to increase C17:0 compared with CO (0.53 vs 0.59 g/100g MFA), with no differences in any other MFA analyzed. In conclusion, supplementation with live yeast improved milk quality by decreasing SCC, even in cows with low SCC, and produced minor changes in milk fatty acid profile.

Key Words: live yeast, dairy cow, milk fatty acid

W99 Meta-analysis of the effect of direct-fed bacteria on intake, digestibility, milk production, and ruminal fermentation of lactating dairy cows.

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The objective of this study was to estimate the magnitude of effects of dietary supplementation of direct-fed bacteria (DFB) on the intake, digestibility, milk production and rumen fermentation of lactating dairy cows. A systematic search in the literature from 2002 to 2018 was conducted and data from 24 peer-reviewed papers with 39 treatments and 480 dairy cows were used for the analysis of raw mean differences (RMD) between DFB supplementation and control. The means were weighted by inverse variance in a mixed model and the heterogeneity was calculated by meta-regression analysis using bacterial strain (*Bacillus* spp., *Propionibacterium* spp., *Enterococcus f*, and *Lactobacillus* spp.), days in milk (DIM) and dose as covariates. Dietary levels of DFB ranged from 2×10^{8} to 2×10^{11} cfu/cow/d. Compared with the control, DFB did not ($P > 0.05$) increase DMI (RMD = −0.01 kg/d [−0.33,0.32]), milk fat (RMD = 0.02% [−0.01, 0.07]), and milk protein (RMD = 0.07% [−0.01 0.03]). However, total-tract digestibility (RMD = 0.72% [0.001, 1.45]), milk yield (RMD = 1.23 kg/d [0.73, 1.73]), and feed efficiency (0.06 [0.03, 0.08]) were increased ($P < 0.05$) by DFB supplementation. Addition of DFB tended to increase ($P = 0.07$) total VFA in the rumen (RMD = 5.12 mM [−0.48, 10.74]) and tended to decrease acetate:propionate ratio (RMD = −0.09 [−0.20,0.01]). High doses of DFB (2×10^{11} cfu/cow/d) increased total VFA concentrations in the rumen compared with low doses (2×10^{10} cfu/cow/d or lower). Compared with the control, *Bacillus* and *Lactobacillus* strains were more effective (RMD = 1.94 kg/d [0.53, 3.36] and RMD = 1.09 kg/d [0.49, 1.68], respectively) at increasing milk production. In conclusion, dietary supplementation of DFB improved rumen fermentation, digestibility, and milk yield in dairy cows

Key Words: direct-fed bacteria, dairy cow, meta-analysis

W100 Comparison of rumen fermentation and microbiome of rumen samples collected via cannula or stomach tube in lactating dairy cows.

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The objective of this study was to compare fermentation profile, and microbial diversity from rumen samples collected using rumen cannula or stomach tube in lactating dairy cows. Three ruminally cannulated lactating dairy cows were used as rumen fluid donors in a 3 × 3 Latin Square design. The experimental period was 28 d and rumen fluid was collected 2 h after feeding on d 21 and d 23 of each experimental period. Treatments were rumen fluid collected from Stomach tube (ST) or from rumen cannula (C). Both samples were collected within 10 min from same animal on same day. The pH of rumen contents was measured immediately after fluid collection and rumen samples were analyzed for volatile fatty acid (VFA), ammonia-N (NH_3-N) concentration, and microbiome analysis. Data were analyzed using GLIMMIX procedure of SAS. Treatments and period were used as fixed factor while cow was used as a random factor. Significance was declared at $P \leq 0.05$. Rumen pH was greater for ST compared with C (6.87 vs 6.25; $P < 0.01$). However, NH_3-N (15.2 vs 10.6 mg/dL; $P < 0.01$) and total VFA (121.8 vs 95.5 m*M*; $P < 0.01$) was greater for C compared with ST. The rumen fluid collection methods had no effects on molar proportion of acetate ($P = 0.21$) and propionate ($P = 0.16$); however, acetate-to-propionate ratio tended to increase with ST compared with C ($P = 0.08$). The majority of sequences obtained with microbiome analysis belonged to Bacterioidetes, Firmicutes, and Cyanobacteria, regardless of the rumen fluid collection method. However, the abundance of Bacteriodetes was greater ($P < 0.01$) while abundance of Firmicutes was lower ($P < 0.01$) for ST compared with C. No effects were observed on the abundance of phylum Cyanobacteria ($P = 0.13$). The rumen fluid collection methods had no effects on Chao 1 ($P = 0.14$) and Shannon index ($P = 0.21$). In conclusion, molar proportion of individual VFA, Chao1, and Shannon index were similar while rumen pH, NH_3-N, and total VFA were affected by the rumen fluid collection method.

Key Words: microbiome, rumen fluid, stomach tube

W101 Nutritional composition of almond hulls for dairy cows.

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Almond hulls (AH) are a common feedstuff in the diet of dairy cattle in California. Commercial AH vary in nutrient composition because of the debris, shell and stick, contamination of the hulls. A survey (Castillo et al., 2012) of 40 dairy farms in California found AH to be an ingredient in 39 out of 104 TMR evaluated with an average feeding rate of 1.5 kg/cow/day and a range of feeding from 0.2 to 3.0 kg. Even though AH are commonplace in diets of lactating cows, there is a paucity of data on the chemical composition and nutritive value of AH. To achieve higher amounts of feeding to lactating dairy cows, more comprehensive information will be required on the nutritive value and chemical quality of AH. The aim of this research was to determine the nutritional composition of different varieties of AH and the impact of debris on composition. Commercial AH were hand sorted to yield pure AH and debris (non-hull material). Twelve samples of commercial AH were studied. Each sample of AH was divided into 2 subsamples. One subsample represented the commercial AH (Total hulls). The other half was hand sorted to separate the hulls (Pure hulls) from non-hull debris (sticks and shells). These samples were then ground for subsequent chemical analysis. Reducing the proportion of Debris in AH reduced the fiber and ash content. The Nonpareil variety was superior in quality as measured by higher sugar content and lower content of ash, lignin, and NDF compared with Other varieties. Crude fiber was more closely associated with aNDFom rather than aNDF suggesting that any change in the legal definition of AH (<15% crude fiber As Is) should be based on NDF content that is ash free. The results from this study will help dairy nutritionists to better formulate diets that include AH for lactating dairy cows to meet their nutritional requirements. Almond huller operations can also use these results to better understand how debris impacts the chemical composition and nutritive value of AH from different almond varieties. This information will also aid the California Department of Food & Agriculture in the decision process to change the law regulating the fiber composition of AH.

Key Words: almond hulls, byproduct

Ruminant Nutrition: Protein/Amino Acids

W102 Effect of Turboviv phytochemicals on soybean meal protein degradation using in vitro method. V. Ballard*[1], F. Robert[1], M. Mireaux[1], and A. Boudon[2], [1]*Groupe CCPA, Janzé, France*, [2]*INRAE, Agrocampus Ouest, PEGASE, Saint-Gilles, France*.

Phytochemicals are known to improve the proportion of diet proteins by-passing the rumen, by binding proteins and limiting their ruminal degradation. This can improve milk yield and milk protein synthesis. The objective of this study was to evaluate the effects of dietary phytochemicals [Turboviv; CCPA, Janze, France] as rumen modifiers on protein degradation with the Ankom Daisy Incubator II System. Three lactating fistulated dairy cows were used for rumen fluid collection in 3 studies. Animals were fed with diets, based on either corn silage or pasture. In each study, rumen fluid was collected at the beginning for control. Then cows were fed during 21 d with phytochemicals, before the 2nd rumen fluid collection (Turboviv treatment). Dry matter (DM, 17 h at 103°C) degradation was determined with samples of alfalfa and soybean meal (46% CP) incubated in a Daisy Incubator II in bags with rumen buffer and rumen fluid for 0, 2, 7, 24, and 48 h. Nitrogen content of the residues (Kjeldahl) was also analyzed for soybean meal. Means were compared within feeds (alfalfa or soybean meal), using GLM function with R considering diets (corn silage or grass), treatments (Control or Turboviv), incubation times and interactions as factors (significance at $P < 0.05$). Turboviv decreased DM degradability of both alfalfa and soybean meal ($P < 0.05$) in rumen fluid related to both diets. The decrease was observed at 2 and 4 h hours of incubation for soybean meal (interaction time × treatments, $P < 0.01$). Turboviv also decreased the protein degradability for soybean meal at 2h and 4h (respectively 4.0% and 10.4%, Table 1). Summary statistics of protein degradation are given in the Table 1. These data suggest that feeding Turboviv can significantly affect rumen fermentation, and more specifically can decrease ruminal protein degradation.

Table 1 (Abstr. W102). Summary statistics of soybean meal protein degradation (%)

	2 h		4 h		7 h		24 h	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	40.8	1.6	54.8	5.9	62.3	8.5	98.1	1.0
Turboviv	36.8	2.4	44.4	5.0	55.7	3.4	97.5	0.9

Key Words: dairy cow, phytochemicals, ruminal degradability of protein

W103 Lactational performance of dairy cows supplemented with *N*-acetyl-L-methionine. S. E. Räisänen*[1], X. Zhu[2,3], C. F. A. Lage[1], M. E. Fetter[1], H. A. Stefenoni[1], A. Melgar[1], D. E. Wasson[1], S. F. Welchez[1], J. S. Eun[4], J. Park[4], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA*, [2]*Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan, China*, [3]*University of Chinese Academy of Sciences, Beijing, China*, [4]*Institute of Biotechnology, CJ Blossom Park, Suwon, Korea*.

The objective of this experiment was to determine the effect of *N*-acetyl-L-methionine (NALM) on milk production and milk composition in lactating dairy cows fed a diet that meets or exceeds the requirements for energy and metabolizable protein (MP) but is deficient in digestible Met (dMet), according to NRC (2001). Eighteen multiparous Holstein cows [60 ± 1.5 d in milk, 638 ± 15 kg BW, 52.8 ± 2.98 kg milk yield (MY)] were used in a replicated 3 × 3 Latin square design experiment with three 28-d periods, balanced for residual effects. Treatments were (1) basal diet supplying 1.66% digestible (d)Met (CON), (2) basal diet supplemented with 32 g/d NALM (NLow), (3) basal diet supplemented with 56 g/d NALM (NHigh). The NALM treatments supplied estimated 16.7 and 29 g/d dMet, respectively. The NALM was top-dressed on the basal diet at morning feeding. Dry matter intake (DMI) and MY were recorded daily, and milk sampling was performed on the last week of each experimental period from 4 consecutive milkings (2, AM and 2, PM). Data were analyzed using PROC MIXED of SAS with treatment and period in the model. Square and cow within square were random effects. Linear effects of NALM dose were tested. Dry matter intake was similar ($P = 0.76$) among treatments (averaging 24.7 kg/d; SEM = 0.72). Milk yield was also not affected ($P = 0.64$) by treatment (averaging 50.7 kg/d; SEM = 1.56), whereas ECM was decreased linearly ($P = 0.03$) by NALM (48.1, 45.1 and 45.0 kg/d, respectively) as a result of a linear decrease ($P = 0.004$) in milk fat concentration (3.77, 3.47, 3.33%, CON, NLow, and NHigh, respectively). Similarly, there was a linear decrease ($P = 0.007$) in milk fat yield with increasing NALM dose: 1.92, 1.74, and 1.69 kg/d for CON, NLow, and NHigh, respectively. The concentration and yield of both lactose and milk true protein were not affected by treatment ($P > 0.10$). In the conditions of this experiment, supplementing NALM to an MP-adequate diet supplying dMet at 1.66% of MP caused a decrease in milk fat and ECM yield and did not affect milk or milk true protein yields in mid-lactation dairy cows.

Key Words: *N*-acetyl-L-methionine, milk production, dairy cattle

W104 Treatment of soybean meal to improve protein utilization by dairy cows. A. Klop[1], M. Aoun[2], J. Ricaud[2], and G. van Duinkerken*[1], [1]*Wageningen Livestock Research, Wageningen, the Netherlands*, [2]*Idena, Sautron, France*.

Soybean meal is a common protein source in dairy cattle diets. By chemical treatment (e.g., with formaldehyde), rumen degradable protein content can be decreased, while increasing ileal digestible protein content. We evauated whether feed additive Vertan (a blend of eugenol, thymol and essentials oils) can be a nature-based alternative for chemical processing of soybean meal. To evaluate the effect of Vertan on performance and nitrogen use efficiency, a feeding trial was conducted with 3 groups of 15 dairy cows each. After a pre-period, cows were grouped and allotted to a treatment: a negative control diet (SBM) with soybean meal (1.4 kg DM/cow/d), a positive control diet (SBM-bp) with formaldehyde treated soybean meal (1.2 kg DM/cow/d) and a treatment diet (SBM-V) with soybean meal supplemented with Vertan (1.4 kg DM/cow/d). Crude protein content of all diets was 15% on a dry matter basis. Parallel to this study, 3 cows fitted with a rumen cannula were used in a Latin square design to measure rumen fermentation characteristics, in situ rumen degradation of the diet and digestion. Total dry matter intake of cows in the performance trial was 22.6 kg/cow/d for all treatments. Milk yield did not differ between treatments. Milk protein content was lower ($P = 0.037$) for SBM-bp (35.7 g/kg) compared with SBM (36.4 g/kg). Blood urea of cows on SBM-V was lower (3.4 mmol/L) ($P = 0.002$) compared with cows on SBM (3.9 mmol/L). Blood urea differences in the performance trial were in accordance with the differences in rumen ammonia concentrations found in the parallel trial with cannulated cows. Ammonia concentration in rumen fluid tended ($P = 0.073$) to be lower for treatments SBM-V (6.9 mmol/L) and SBM-bp (7.0 mmol/L) compared with SBM (9.1 mmol/L). The total VFA concentration in rumen fluid was highest ($P = 0.014$) for SBM (117.5 mmol/L) compared with SBM-bp (101.5 mmol/L) and SBM-V (104.7 mmol/L). Rumen pH tended ($P = 0.055$) to be higher for SBM-bp (6.25) compared with SBM (6.14) and SBM-V (6.18). Apparent total-tract digestibility did not differ between treatments, Vertan had no effect on DM, CP, NDF and starch digestibility. We concluded that the use of Vertan affects rumen protein fermentation, resulting in lower rumen ammonia and blood urea.

Key Words: protein, nitrogen, utilization

W105 Evaluation of an underivatized compared with a derivatized method to quantify bovine plasma amino acids via liquid chromatography electrospray mass spectrometry. M. Z. Toledo*[1],

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Objectives were to evaluate and compare a novel underivatized with a derivatized method to quantify bovine plasma AA by isotope dilution ratio via liquid chromatography electrospray single quadrupole mass spectrometry. Plasma from lactating dairy cows (n = 24; 80 DIM; 49 kg/d of milk) was isolated by centrifugation and deproteinized in 0.5 *N* perchloric acid. Deproteinized plasma was filtered and injected into a 50 × 2 mm column (Imtakt, OR) or extracted, derivatized, and injected into a 250 × 3 mm column (EZ:Faast, Phenomenex, CA) and analyzed. Linearity of the mass-to-charge ratio (m/z) signal was evaluated for each AA with 5-point standard curves (range: 3.9 to 500 μ*M*). Recovery rates were evaluated using pooled plasma samples (n = 4) spiked with 10, 20, or 50 μ*M* of each AA. Statistical analysis was performed using the TTEST and IML procedure of SAS. Both methods showed linearity within the dynamic range analyzed for all essential AA ($R^2 \geq 0.995$). Meanwhile, underivatized samples had poor chromatogram (signal-to-noise ratio < 3) or linearity ($R^2 \leq 0.990$) for cystine, Asp, Gly, Tyr, and Ser. Moreover, sensitivity for ^{12}C AA was greater for derivatized than underivatized samples. Both methods had recovery rates ranging from 84.5% to 126.0% and none differ from 100% ($P \geq 0.05$), except Gln (20 μ*M* [85.7%] and 50 μ*M* [87.6%], $P < 0.01$) and Val (50 μ*M* [120.6%], $P < 0.02$) using the underivatized method. Coefficient of correlation (r) and concordance coefficient of correlation (CCC) varied from 0.42 to 0.99. Highest r and CCC was observed for Arg (r = 0.99; CCC = 0.99), Leu (r = 0.98; CCC = 0.98), Met (r = 0.99; CCC = 0.96), and Thr (r = 0.97; CCC = 0.96). In particular, the Bland-Altman plot showed that Met has a mean bias of 2.1 μ*M*. In conclusion, derivatization had greater ^{12}C AA sensitivity, linearity, and recovery rates, however, the underivatized method showed linearity and accurate recovery rates for most essential AA, and in particular Met quantification appears to be comparable with a derivatized method.

Key Words: amino acids, bovine, mass spectrometry

W106 Effect of feeding rumen-protected methionine pre- and postpartum on health, herd exit dynamics, and time to pregnancy in multiparous dairy cows. M. Z. Toledo*[1], M. L. Stangaferro[2], R. S. Gennari[1], P. L. J. Monteiro Jr.[1], M. M. Perez[2], M. Masello[2], M. E. Van Amburgh[2], D. Luchini[3], R. D. Shaver[1], J. O. Giordano[2], and M. C. Wiltbank[1], [1]*University of Wisconsin-Madison*, *Madison, WI*, [2]*Cornell University*, *Ithaca, NY*, [3]*Adisseo*, *Alpharetta, GA*.

Objectives were to evaluate the effect of feeding rumen-protected methionine (RPM) from 3 wk prepartum up to 147 d postpartum on clinical health disorders, uterine health, herd exit dynamics and time to pregnancy in multiparous dairy cows. A total of 470 multiparous Holstein cows [235 cows at University of Wisconsin (UW) and 235 cows at Cornell University (CU)] were enrolled prepartum, fed a basal diet, and randomly assigned treatments: Fed RPM (Smartamine M; MET), UW:2.83% and 2.58%, CU: 2.85% and 2.65% of Met as % of metabolizable protein (MP) and control (CON) = UW:2.30% and 2.09%, CU:2.22% and 2.19% of Met as % of MP, respectively pre- and postpartum. Cows were monitored for health disorders during the feeding experimental period and herd exit dynamics and time to pregnancy were evaluated up to 300 DIM. Cytological endometritis was evaluated at 35 ± 3 DIM. Cows received first AI after Double-Ovsynch at 80 ± 3 DIM followed by systematic reproductive management. Pregnancy diagnosis was performed by transrectal ultrasound (32 d after AI). Health data were analyzed by logistic regression and pen was the experimental unit. Cox's proportional hazards regression was used for the analysis of time to event. Treatments had no effect on proportion of cows with displaced abomasum, ketosis, mastitis, retained placenta, respiratory problems or lameness ($P > 0.10$). However, cows fed MET tended ($P = 0.08$) to be less likely to be sold (16.9% vs 10.7%) and had lower risk of being sold up to 300 DIM than CON (hazard ratio [HR] = 1.59, CI = 0.97 to 2.62, $P = 0.07$). No effects were observed on first service Pregnancy/AI or pregnancy loss ($P > 0.10$). Overall, cows fed MET tended to have reduced time to pregnancy (HR = 1.14, CI = 0.93 to 1.39, $P = 0.10$). Moreover, within cows with multiple health disorders (n = 116), cows fed MET had shorter time to pregnancy than CON (HR = 1.46, CI = 0.97 to 2.20, $P = 0.04$). In conclusion, feeding MET to multiparous cows pre- and postpartum may reduce time to pregnancy, particularly in cows with multiple health disorders, and appears to decrease likelihood of cows being sold.

Key Words: methionine health pregnancy

W107 Elucidating the effects of branched-chain amino acid availability on lactose synthesis rates using mammary tissue slices and isotope-labeled glucose. J. V. V. Silva*[1], L. Showman[2], H. K. J. P. Wickramasinghe[1], N. Stepanchenko[1], M. J. O. Hidalgo[1], M. A. Perera[2], and J. A. D. R. N. Appuhamy[1], [1]*Department of Animal Science, Iowa State University*, *Ames IA*, [2]*W. M. Keck Metabolomics Research Laboratory, Iowa State University*, *Ames IA*.

A meta-analysis revealed even though branched-chain amino acid (BCAA) supplementation tended to increase milk yield, plasma leucine (Leu) concentration was negatively related to lactose yield in lactating dairy cows. The objective of this study was to investigate the effects of extracellular BCAA availability on lactose synthesis rates using bovine mammary tissue slices (MTS) and stable isotope-labeled glucose. Mammary tissue slices (119 ± 21 mg) were obtained from 3 lactating Holstein cows slaughtered on 3 separate days. Cows were free of mastitis and in late lactattion. The MTS were incubated in 1) medium with all essential amino acids at physiological concentrations (+EAA), 2) +EAA supplemented with Leu at 10 × the physiological concentration (2.0 mM, +LEU), 3) +EAA deprived of all BCAA (-BCAA), and 4) +EAA deprived only of Leu (-LEU) for 3.0 h (t = 3.0). All media contained [$^{13}C_2$]-glucose at 3.15 mM as the sole source of glucose (100% extracellular [$^{13}C_2$] enrichment). Homogenates of cultured tissue slices were subjected to a gas chromatography-mass spectrometry analysis to determine [$^{13}C_2$] enrichment in intracellular precursors (ICE) and single moiety-labeled lactose (LCE). The ICE and LCE were then used to calculate fractional synthesis rate of lactose [FSR, %/h = (LCE × 100)/ (ICE × t)]. Treatment effects on ICE, LCE, and FSR were analyzed with a mixed-effect model including fixed effect of treatment and random effect of cow using the MIXED procedure of SAS. Intracellular precursor enrichment was similar among treatments at 82 ± 2% ($P = 0.840$). Mean LCE was 12.0 ± 1.1% across treatments. The LCE of –BCAA ($P = 0.023$) and –LEU ($P = 0.080$) were lower than that of +LEU. Mean FSR was 5.0 ± 0.7%/h across treatments. Fractional lactose synthesis rates of –BCAA tended to be lower than that of +LEU (5.8 vs. 4.4%/h, $P = 0.062$). Overall, extracellular availability of total BCAA or only Leu appeared to favorably affect lactose synthesis rates in bovine mammary tissues. The MTS together with stable isotope-labeled glucose could provide a good model for studying how lactose synthesis rates are regulated.

Key Words: leucine, lactose synthesis, bovine mammary

W108 Effect of rumen-protected methionine supplementation and its relationship with lipid metabolism in high-producing dairy cows with different dietary energy concentrations. J. H. Carneiro*[1], D. R. Ribeiro[1], E. J. Askel[1], I. F. Carrari[1], M. Poczynek[1], F. Lopes[2], J. S. Osorio[3], and R. Almeida[1], [1]*Universidade Federal do Paraná*, *Curitiba, PR, Brazil*, [2]*Adisseo South America*, *São Paulo, SP, Brazil*, [3]*South Dakota State University*, *Brookings, SD*.

A post-absorptive link between amino acid supplies and lipid metabolism has been suggested and evidenced. The objective of this trial was to determine the effect of methionine (MET) supplementation at 2 levels of dietary ME on the performance of mid-lactation dairy cows. A total of 112 multiparous Holstein cows (49.4 ± 5.3 kg/d of milk and 146.6 ± 68.2 DIM) from a commercial herd in Southern Brazil were blocked by lactation number,

Table 1 (Abstr. W108). Effect of methionine and energy concentration on milk production and composition

Item	CON	HE	MET	HE+MET	SEM	*P*-value
Met (%MP)	2.02	2.02	2.40	2.40		
Lys:Met	3.20	3.20	2.71	2.71		
Met:ME	0.87	0.84	1.03	1.00		
Milk yield, kg/d	48.5^{ab}	49.7^{a}	47.7^{b}	49.6^{a}	0.24	0.01
ECM, kg/d	50.0^{b}	52.4^{a}	52.0^{ab}	53.7^{a}	0.28	<0.01
Fat, %	3.82^{b}	3.92^{ab}	4.16^{a}	4.12^{a}	0.03	<0.01
Total protein, %	3.16^{c}	3.15^{c}	3.27^{b}	3.34^{a}	0.01	<0.01
Lactose, %	4.64^{ab}	4.64^{ab}	4.60^{b}	4.65^{a}	0.01	0.03
Fat, kg/d	1.84^{b}	1.95^{ab}	1.99^{a}	2.03^{a}	0.01	<0.01
Total protein, kg/d	1.51^{c}	1.57^{b}	1.56^{b}	1.65^{a}	0.01	<0.01
Lactose, kg/d	2.24^{ab}	2.31^{ab}	2.20^{b}	2.31^{a}	0.01	0.03
MUN, mg/dL	12.6^{b}	14.1^{a}	12.9^{b}	14.1^{a}	0.10	<0.01

milk yield, and DIM. Cows were divided into 4 isoprotein (16.6% CP) diets: control (CON; 1.62 NE_L Mcal/kg DM); high energy (HE; CON +1.2% of DM of hydrogenated fat (Prius F 100 Nat Dry, Auster)); methionine (MET; CON + 24 g of Smartamine M, Adisseo SA); and HE+MET (HE + 24 g of Smartamine M). The total experimental period was 38 d, and the first 3 d were used as a covariate with all cows fed the CON diet. Total mixed ration was provided once a day, with or without the addition of HE treatment, while MET treatments were top-dressed twice a day. Milk yield data were recorded 3 times a day, while milk samples for composition were collected weekly, as well as in the last 9 consecutive milkings of the experimental period for composition, fatty acid profile, and gene expression. Data were analyzed using the MIXED procedure of SAS with repeated measures. The greater supply of MET showed the potential to alter lipid and protein metabolism, enhanced with an energy-dense diet (Table 1).

Key Words: amino acid, energy, fat

W109 An 8-hour hyperinsulinemic-euglycemic clamp is insufficient to alter milk production in lactating dairy cows. V. L. Pszczolkowski[1,2], H. Hu[2], J. Zhang[2,3], M. K. Connelly[2], A. S. Munsterman[4], and S. I. Arriola Apelo*[2,1], [1]*Endocrinology and Reproductive Physiology Graduate Training Program, University of Wisconsin-Madison*, *Madison, WI*, [2]*Department of Animal and Dairy Sciences, University of Wisconsin-Madison*, *Madison, WI*, [3]*Department of Animal Science, China Agricultural University*, *Beijing, China*, [4]*Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison*, *Madison, WI*.

The hyperinsulinemic-euglycemic clamp (IC) technique is an important tool to study energy metabolism in a variety of species. In dairy cattle, IC is usually performed continuously over several days, which can result in decreased intake. Since intake and insulin secretion are not naturally continuous in cows but rather follow a diurnal rhythm, our objective in this study was to test whether a single 8-h IC would be sufficient to observe milk production changes in response to IC and to insulin-responsive amino acids. Six second-lactation ruminally cannulated Holstein cows (155 ± 9 DIM) were used in a 2 × 2 Latin square design (7-d periods). Cows were fed a 20% MP-restricted diet, and abomasally infused with water (8 L/d) or Met (26 g/d) plus Leu (70g/d, ML) dissolved in the same volume water. Abomasal infusions were administered for 8 h/d. On d 6, cows were fitted with jugular catheters. Feed intake and refusals were measured daily. On d 7, IC was performed as infusion of 1ug/kg/hr insulin, with euglycemia maintained by varying glucose (50%wt/vol in saline) infusion rate based on tail vein blood glucose concentration; saline (0.9% NaCl, SAL) was infused at same volume rate than insulin (110 mL/h). Milk samples were taken on d 7 p.m. Data were analyzed by 2-way ANOVA for main effects and interaction of AA and IV. Dry matter intake on d 7 was decreased by IC during the infusion period by 7 kg ($P = 0.02$), but this difference was recovered by post-IV intake ($P = 0.45$). Blood glucose was lower for IC by 8.2 mg/dL at 4 h, and the difference was maintained until the end of infusion ($P = 0.03$). Milk yield was numerically increased by IC (3 kg, $P = 0.14$), as was protein yield (60 g, $P = 0.19$), but fat was unaffected. Lactose yield was numerically higher for IC ($P = 0.22$), but percent was lower ($P = 0.03$). Amino acid infusion had no effect on any response except for increasing MUN ($P = 0.07$). Despite our preliminary data suggests that insulin mediates the effect of TOR-AA on milk production, 8 h IC seems insufficient to achieve that effect.

Key Words: insulin clamp, amino acids, milk production

W110 Durability evaluation of a rumen-protected choline supplement during mill and farm handling. H. Diaz*, M. Sellers, S. McGregor, J. Albrecht, O. Drehmel, and J. Linn, *Milk Specialties Global*, *Eden Prairie, MN*.

A novel lipid coated rumen-protected choline (RPC) supplement was evaluated for handling and mixing durability in a commercial feed mill and on farm. The RPC contained 24% choline chloride and was coated with saturated fatty acids. Means were compared using JMP and Students *t*-test with significance at $P < 0.05$. For this study, 129 kg of RPC was delivered to the[JL1] feed mill in MN, added to 1,361 kg of grain/mineral mixture (RPC-MIX) and mixed for 3 min in a ribbon mixer and stored in a vertical bin at the mill. The next day RPC-MIX was transferred to an auger truck, delivered to a commercial dairy and stored in a vertical bin. RPC-MIX was removed daily from the bin and mixed into a TMR for feeding. Samples of RPC were collected (1) before mixing at the mill (RPC-CON), (2) after mixing with grain/mineral and before storage in the mill (RPC-MIX), and (3) on farm at the feed bunk after mixing into a TMR with forages and other concentrates (RPC-TMR). Durability was determined by testing crude protein (CP) in rumen undegradable protein (RUP) and intestinal digestibility (ID). RPC-MIX and RPC-TMR samples were sieved for isolation and collection of RPC. For RUP, samples were incubated in the rumen of 2 cannulated cows for 16 h and reported as % of undigested CP. RUP was 97.0, 91.3 and 87.0 for RPC-CON, RPC-MIX and RPC-TMR and not different among sampling times ($P > 0.24$, SEM = 3.90). ID (% of CP) of RPC was determined using the Cornell method for in vitro intestinal digestibility. The ID for RPC-CON and RPC-MIX and RPC-TMR was 85.3, 68.2 and 70.5% ($P = 0.06$, SEM = 3.15), respectively. ID was highest in RPC, declined slightly with mixing in the mill, but not further declined with transfer to and mixing on the farm. [JL2] The RPC as manufactured has a high initial durability as shown by the high RUP and ID of the RPC-CON. Mill handling and mixing had no effect on RUP, but tended to decrease ID compared with RPC-CON. Durability of RPC was not affected by on farm storage and TMR mixing. The data indicate a saturated fatty acid coating can be very effective in protecting choline against rumen degradation and achieving good intestinal digestibility.

Key Words: choline, RUP, digestibility

W111 Plasma amino acid response to casein or acid casein hydrolysate in Holstein calves.

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Previous reports in cows suggest that AA response to enzymatically hydrolyzed casein and casein are similar, but plasma AA concentrations in nonruminants increase in amount and rate to hydrolyzed protein compared with complete protein. Typically, degree of hydrolysis in enzymatically hydrolyzed casein is less than the degree of hydrolysis in acid hydrolyzed casein (AHC). We fed AHC and sodium caseinate (CAS) to 6 Holstein bull calves (initial BW = 54 ± 1.4 kg) to compare bioavailability of AHC and CAS. Calves were provided either 0, 60, or 120 additional grams of AAs from either AHC or CAS added to a milk replacer (403.1 ± 0.73 g DM) formulated to meet or exceed ME and Lys requirements of a 68 kg calf gaining 0.45 kg daily. Calves were adapted to treatments for 6 d and samples of jugular blood were collected each 4 h on d 7 to determine AA bioavailability. Each calf was provided each combination of treatments balanced for carryover effect. Plasma essential AA increased linearly ($P < 0.01$) with increasing intake of AHC and CAS, but rate of increase among plasma essential AA in response to greater intake of AHC and CAS was similar ($P = 0.28$). Branched-chain amino acids accounted for over 70% of increases in essential AA regardless of source of AA. Lysine tended to increase linearly in response to AHC ($P = 0.06$) while there were no changes in concentration of Thr or Trp in response to AHC or CAS ($P \geq 0.18$). Concentrations of nonessential AA increased linearly with AHC ($P < 0.01$) but only tended to increase in response to CAS ($P = 0.06$). Rate of increase in nonessential AA to AHC was greater (1.42 μM/g AA) compared with CAS (0.62 μM/g AA, $P = 0.02$). Glutamate, Asp, Ala, Ser, and Pro increased at a greater rate ($P \leq 0.04$) in AHC fed calves compared with calves fed CAS while Tyr, Asn, and His increased at a greater rate ($P \leq 0.04$) in CAS compared with AHC. Plasma concentrations of Gly decreased ($P = 0.01$) with increasing sodium caseinate intake. Rate of increase in total plasma AA concentration in response to AHC tended ($P = 0.11$) to be 145% greater than CAS. These data seem to indicate that AA bioavailability it greater to AHC than to CAS in milk-fed calves.

Key Words: amino acid, bioavailability, casein

W112 Estimated postruminal bioavailability of rumen-protected lysine using plasma area under the curve methodology.

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Products purported to provide rumen-protected Lys (RP-Lys) must be evaluated for postruminal delivery of Lys for absorption and metabolism. The objective of this study was to determine the relative bioavailability (BA) of intestinal absorption for RP-Lys using the plasma area under the curve (AUC) methodology. Three lactating rumen-cannulated Holstein dairy cows weighing 724 ± 28 kg, producing 31.4 ± 5.1 kg/d of milk were used in a 3 × 3 Latin square design. Each period consisted of 6 d of washout and 1 d of infusion and sampling. Cows were fed a basal TMR diet formulated to meet or exceed protein, Lys, and Met requirements. A PVC tube was temporarily inserted through the rumen cannula into the omasum and treatments (TRT) were infused through the tubing. The positive control was 58.9 g of crystalline HCl-Lys, assumed to be 100% absorbed, diluted in 300 mL of water. The same amount of Lys in a RP form was infused (150 g - 39.3% of Lys, ± 0.9), and the sham control was an abomasal infusion of 360 mL of water. Lysine was measured in blood samples collected before and at 30, 60, 90, 120, 240, 360 and 480 min following the infusions. Plasma Lys absorption was estimated from the AUC with respect to time. Data were analyzed using the MIXED procedure of SAS with the fixed effects of TRT and period and random effects of cow within TRT. The relative BA for RP-Lys was determined as the ratio of the RP-Lys AUC over the free HCl-Lys AUC after subtracting the AUC value from the water infusion. Postruminal dosing with HCl-Lys resulted in a greater AUC when compared with RP-Lys and water (354.7, 135.5, and 71.2 ± 16.95 $\text{nmol}\cdot\text{min}\cdot\text{mL}^{-1}$ for HCl-Lys, RP-Lys and water, respectively). The relative BA of the RP-Lys product was 22.7%. There was a trend ($P = 0.08$) for greater Lys half-life in the plasma for RP-Lys relative to water infusion (546, 853, −886 ± 424.1 min for HCl-Lys, RP-Lys and water, respectively). Plasma Lys clearance rate with HCl-Lys was 0.16 ± 0.1% min^{-1} between infusions. The results indicated that the RP-Lys product was able to deliver Lys and be absorbed in the small intestine.

Key Words: rumen-protected lysine, plasma lysine, dairy cow

W113 Can ε-polylysine provide metabolizable lysine to cattle?

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Metabolizable Lys can limit growth or lactation in cattle fed corn-based diets. Addition of crystalline Lys to diets does not provide large increases in metabolizable Lys because it is ruminally fermented, and efforts to increase metabolizable Lys with lipid coatings seem to provide only slight increases in metabolizable Lys flows to cattle. ε-Polylysine (εPL) is a peptide comprised of Lys residues with antimicrobial activity and is commonly used to preserve foods and cosmetics. Thus, εPL may be an effective ingredient to increase metabolizable Lys in diets fed to cattle. Our objective in this experiment was to determine if εPL was able to provide metabolizable Lys to cattle. We used 6 Holstein bull calves (initial BW = 52 ± 2.2 kg) to evaluate the bioavailability of εPL compared with Lys-HCl. Each calf was provided 0, 4.8, or 9.6 g of Lys by addition of Lys-HCl or εPL to a milk replacer (403.11 ± 0.58 g DM / d) formulated to meet or exceed the ME and metabolizable Lys requirements of a 68 kg calf gaining 0.45 kg daily. Each calf was provided each combination of treatments in a manner balanced for carryover effect. Calves were adapted to treatments for 6 d and samples of jugular blood were collected each 4 h on d 7 to determine AA bioavailability. Data were analyzed using the MIXED procedure of SAS and differences in Lys bioavailability were determined from the ratio of the slopes between Lys-HCl and εPL. Plasma Lys concentrations increased linearly in response to greater Lys intake from Lys-HCl (slope = 13.51 μM/g Lys, $P < 0.01$), but plasma Lys concentrations did not respond to increased intake from εPL ($P = 0.29$). Nonetheless, increases in plasma Lys in response to greater εPL intake resulted in a 15% numerical increase in Lys bioavailability from εPL. Plasma concentrations of Thr, Met, Glu, and Gln decreased ($P \leq 0.03$) with increased εPL intake while concentrations of His, Val, Leu and Ile increased ($P \leq 0.01$) with increased εPL intake. Apparently, εPL is not a good source of metabolizable Lys to calves. Concentrations of other AA were affected by εPL; perhaps as a result of altered luminal transport of AA in response to the strong cationic characteristics of εPL.

Key Words: cattle, lysine

W114 Energy source conditions the milk response to TOR amino acids in dairy cows.

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Table 1 (Abstr. W115).

	Treatment			*P*-value		
Item	CMR	SMR	SE	Treatment	Time	Treatment × Time
Milk replacer, g/d	1,038	1,035	4	0.62	<0.001	0.13
Starter, g/d	533	503	50	0.69	<0.001	0.12
Hay, g/d	77	69	6	0.36	<0.001	0.26
Weight (wk 8), kg	87.2	86.2	1.5	0.65	—	—
Weight (wk 10), kg	102.2	102.0	1.7	0.93	—	—
ADG (wk 1–10), g/d	890	874	17	0.53	<0.001	0.39
Gain:feed (wk 1–9), kg/kg	0.65	0.63	0.01	0.28	<0.001	0.87
Serum Lys, mg/mL	30.7	29.6	0.9	0.41	<0.001	0.62
Serum Met, mg/mL	15.9	13.9	0.6	0.03	<0.001	0.02
Serum Ile, mg/mL	17.4	22.9	0.5	<0.001	<0.001	0.16
Serum Leu, mg/mL	27.2	35.5	0.8	<0.001	<0.001	0.27
Serum Val, mg/mL	31.2	48.1	0.9	<0.001	<0.001	0.01

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TOR-AA are those amino acids that stimulate the mechanistic target of rapamycin complex 1 (mTORC1), including Leu and Met. Insulin is required for TOR-AA stimulation of mTORC1. The objective of this study was to determine if energy source affects milk production response to TOR-AA and nonTOR-AA. We hypothesized that glucogenic energy, through stimulation of insulin, potentiates the effects of TOR-AA on milk protein synthesis in dairy cows. Six second lactation, ruminally cannulated Holstein cows (93 ± 9 DIM) with the right-side carotid artery transposed to subcutaneous level were used in a 6 × 6 Latin square design with 12-d periods. Cows were fed a basal diet restricted in ME and MP by 11 and 30%, respectively, and supplemented with a rumen inert fat (FAT, 563 g/d) or abomasally infused with an isocaloric amount of glucose (GLUC, 1 kg/day). Under each energy background, cows were abomasally infused with water (CTRL, 8 L/d), the TOR-AA (Met 30 g/d, Leu 80 g/d, TOR), or the nonTOR-AA (His 20g/d, Lys 60 g/d, NTOR). Infusions were administered for 8 h/d. Feed intake and refusals were measured on d 9 and 10; composite milk samples were collected on d 9 p.m. through d 11 a.m. (4 milkings); timed serial blood samples were taken on d 11. Data were analyzed by 2-way ANOVA; means were separated by pre-planned contrasts adjusted by Bonferroni. Dry matter intake was unaffected by treatment ($P > 0.1$). GLUC increased arterial insulin by 0.3 ug/L ($P < 0.001$). There was an interaction between AA and energy for milk protein, lactose and de novo fat yield ($P < 0.5$). GLUC increased milk protein yield by 70 g/day as compared with FAT under TOR-AA ($P < 0.047$) but had no significant effect under CTRL or NTOR ($P > 0.1$). Surprisingly, GLUC also improved lactose (240 g/d, $P < 0.001$) and de novo fat (61 g/d, $P < 0.001$) yields under TOR, and de novo fat under NTOR (45 g/d, $P = 0.003$). In conclusion, GLUC mediated the milk component synthesis response to TOR-AA, suggesting an mTORC1 mediated effect of glucose or more specifically insulin and TOR-AA on milk production.

Key Words: amino acids, energy, mechanistic target of rapamycin complex 1 (mTORC1)

W115 Evaluation of branched-chain amino acid inclusion in milk replacers on growth and health of Holstein calves. S. Y. Morrison*[1], H. Gauthier[1], A. Obata[2], K. Hirano[2], and H. Uchihori[2], [1]*William H. Miner Agricultural Research Institute, Chazy, NY*, [2]*ZEN-NOH National Federation of Agricultural Cooperative Association, Tokyo, Japan.*

Branched-chain amino acids (BCAA; Leu, Ile, and Val) are required in the diet but evaluation of BCAA in calf diets is limited. The objective of this study was to evaluate the effect of including BCAA in calf milk replacer (MR) on calf growth and health through 10 wk of age. Female Holstein calves (n = 52) were blocked by birth date and assigned to 1 of 2 milk protein-based MR formulated to contain 26% crude protein 18% fat, 2.34% Lys, and 0.72% Met (dry basis) in a complete block design. Treatments were a control MR (CMR) or a MR with additional BCAA included at concentrations observed in whole milk [SMR; Leu (2.81%), Ile (1.68%), and Val (1.89%)]. Intake of MR (wk 1–8), starter (wk 2–9), and hay (wk 7–9) and health scores were recorded daily through wk 9 of age. Body weight and stature measurements were recorded weekly through 10 wk of age. Blood was sampled at wk 2, 6, and 8 of age. Data were analyzed using the MIXED and GLIMMIX procedures in SAS. The model included the fixed effect of treatment, time, and block. Initial measures were used as a covariate when appropriate. Results are shown in Table 1. Initial BW (42.1 ± 0.7 kg) and serum total protein (6.4 ± 0.1 g/dL) were not different between treatments. No difference in intake, weight, and body stature measurements or fecal, respiratory, or hydration scores was observed between treatments. Additional BCAA in MR did not influence growth or health of calves but did decrease serum Met concentration and increase serum BCAA concentrations in the preweaning period.

Key Words: branched-chain amino acid, milk replacer, calf

W116 Performance and metabolic responses to rumen-protected methionine supplementation in lactating dairy goats fed two levels of metabolizable protein diets. L. Bahloul*[1] and P. Schmidely[2], [1]*Centre of Expertise and Research in Nutrition, Adisseo France S.A.S, Commentry, France*, [2]*Université Paris-Saclay, INRAE, AgroParisTech, UMR Modélisation Systémique Appliquée aux Ruminants, Paris, France.*

The objective was to evaluate the performance of alpine goats to 2 dietary CP levels supplemented with rumen protected methionine (RPM). Sixteen multiparous alpine goats (88.5 ± 5.5 DIM) were randomly allocated in individual pen according to their milk CN-S1 genotype (High vs Low), BW, DIM, milk yield (MY) and milk protein content (MPC), and randomly assigned to a Low CP (LP,14%CP) vs High CP level (HP,16%CP) without RPM (Smartamine M, Adisseo) or with RPM at rate of 0.12%DM (+RPM) to cover 100% of metabolizable methionine (MetDi) requirement based on cow requirement (INRA, 2007). The trial was a crossover split-plot design for 9 wk: 2 periods of 4 wk each separated by 1 washout week. Milk and blood samples were collected before the morning feeding twice and once per week, respectively. Data were analyzed with MIXED procedure of SAS. DMI did not differ between groups. Goats fed RPM had higher MY in LP diet (3.98 vs 4.08 kg/d, CP×RPM: $P = 0.02$). Fat content did not differ between groups. Goats fed RPM increased MPC when fed HP diet (3.11 vs 3.21, CP×RPM: $P < 0.01$) especially in goats having Low CN-S1 (CN-S1×RMP: $P = 0.10$). Milk protein yield (MPY) did not differ between groups. Casein content increased in goats with high CN-S1 (23.6 vs 26.9 g/L, $P = 0.02$). Milk fat yield (MFY) increased in LP+RPM diet (117 vs 124 g/d, CP×RMP: $P = 0.05$). Lactose content did not differ between groups. Goats fed RPM had higher plasma BHB (0.63 vs 0.59 mM, $P = 0.05$), tended to have higher plasma Met (33 vs 37 μM, $P = 0.10$) and had lower serine (129 vs 115 μM, $P = 0.03$) and tyrosine (75 vs 67 μM, $P = 0.04$). Goats fed HP diet had higher plasma urea (0.49 vs 0.63 g/L, $P < 0.01$). Increasing CP did not alter plasma AA except for higher arginine (180 vs 206 μM, $P = 0.05$). In summary, increasing CP level did not improve performances of lactating dairy goats. An excess of MP supply could explain the poor MPY responses in Met balanced diets.

Key Words: amino acid, milk composition, dairy goats

W117 Variations in protein digestibility of blood meal, feather meal, and a rumen-protected lysine prototype within and across laboratories. K. A. Estes*[1,2], P. S. Yoder[1,3], C. Stoffel[4], and M. D. Hanigan[1], [1]*Virginia Tech*, *Blacksburg, VA*, [2]*Balchem Corporation*, *New Hampton, NY*, [3]*Perdue AgriBusiness LLC*, *Salisbury, MD*, [4]*Papillon Agriculture Company*, *Easton, MD*.

The objectives of this work were to (1) compare post-ruminal protein availability estimates from 2 different in vitro procedures (Ross 3-step and pepsin digestibility assays) with in vivo observations for blood meal (BM), feather meal (FM) and a rumen protected lysine prototype (RP-Lys), and (2) to assess the in vitro assays variability for those ingredients within and across laboratories. Three commercial laboratories each received subsamples of BM (n = 14) and FM (n = 22) for assessment of CP, rumen undegradable protein (RUP), and digestible RUP (dRUP). One laboratory received subsamples of the RP-Lys prototype (n = 5). The supply of AA from BM and FM were evaluated within CNCPS v6.55 using a lactating diet and BM and FM inputs derived from the CNCPS feed library, in vivo, or in vitro results. The effect of laboratory on in vitro protein digestibility was assessed using the GLM procedure in SAS 9.3 and post-test comparisons of the means were made using the LSMEANS statement with the HSD option. Across laboratories, differences ($P < 0.0001$) were observed for BM dRUP where Lab 1 reported a lower estimate (67%) than Lab 2 (93%) or Lab 3 (92%). Also, for FM dRUP, Lab 1 (40%), Lab 2 (34%) and Lab 3 (73%) all reported different values ($P < 0.0001$). Within laboratories, BM estimates were reported in a narrow range, suggesting low procedural variation. But, when testing multiple subsamples of FM or the RP-Lys prototype within a laboratory, CV values ranged up to 11% and 34%, respectively. In vitro dRUP estimates for BM from one laboratory closely matched those determined in vivo (67 vs. 65%, respectively), but no in vitro estimates for FM were similar to in vivo values. For the RP-Lys prototype, dRUP estimates from the in vitro method were half of that determined by the in vivo methods suggesting poor in vitro and in vivo procedural correlation for these ingredients. In vitro methods should be refined and evaluated for accuracy and precision for all ingredients and standardized across laboratories. This effort will result in more accurate model inputs for ration balancing leading to better animal performance.

Key Words: in vitro, blood meal, feather meal

W118 Identification of dietary and physiological factors potentially impeding responses of lactating dairy cows to dietary fortification in metabolizable methionine: A meta-analysis. L. Bahloul*[1] and C. Loncke[2], [1]*Centre of Expertise and Research in Nutrition, Adisseo France S.A.S*, *Commentry, France*, [2]*Université Paris-Saclay, INRAE, AgroParisTech, UMR Modélisation Systémique Appliquée aux Ruminants*, *Paris, France*.

This study aimed to establish response equations on milk protein content (MPC) and milk protein yield (MPY) to rumen protected methionine (RPM) supplementation and to determine response variation variables (interfering factors). Meta-analyses were applied using Minitab (v.17) software on published data (25 experimental groups and 36 treatments) using RPM as Smartamine M or Metasmart (Adisseo), selected from RPM database of peer reviewed publications. Diets were characterized according to INRA 2007 feeding system for dairy. Lactating dairy cows consumed on average 20.3 ± 4.1kg DM, 172 ± 18.2g CP/kg DM and 1643 ± 70MJ NEl/kg DM. Robust within-study models were obtained to predict MPC ($R^2_{adj} = 0.94$, RMSE = 0.04), and MPY ($R^2_{adj} = 0.98$, RMSE = 32.4g/d) to RPM based on the metabolizable methionine expressed as % metabolizable protein (MetDi %PDI). The MPC model suggested that an increase of 0.7%MetDi induced an increase of 0.13%MPC. The within-study slopes were positively affected by % concentrate, HisDi, digestible OM and NE_L ($P < 0.1$). For a same %MetDi, the MPC increased with GluDi content ($P < 0.05$) and decreased with fat content ($P < 0.1$). The MPY model indicated an average increase of 72g and an increase of 104 and 40g MPY/d for an increase of 1%MetDi in early and middle lactation. The within-study slopes were positively affected by DMI, and starch content ($P < 0.05$) and negatively by fermentable OM and IleDi content ($P < 0.05$). For a same %MetDi, the MPY increased with DMI, starch, ME, NE, and RUP content ($P < 0.01$) and decreased with fat content, fermentable OM, LysDi, ThrDi, ValDi, IleDi, PheDi, AspDi, SerDi, GlyDi and AlaDi content ($P < 0.01$). In summary, this work showed that is possible to predict the responses of lactating dairy cows as function of RPM and figure out interfering factors from diets that could affect these responses. These results provide deeper insight to biology processes and may help improving dietary formulation.

Key Words: meta-analysis, amino acids, ruminant

W119 Effects of differing dietary starch and digestible amino acid supply on amino acid efficiency and lactation performance in dairy cattle. P. A. LaPierre*[1], S. Fredin[2], D. A. Ross[1], and M. E. Van Amburgh[1], [1]*Cornell University*, *Ithaca, NY*, [2]*Adisseo*, *Alpharetta, GA*.

Efforts to optimize nitrogen (N) efficiency and improve AA balancing in lactating dairy cattle, particularly when describing the supply and requirements of essential AA (EAA), have demonstrated a relationship between metabolizable AA supply (AAS) and metabolizable energy (ME). Additional data demonstrated that gluconeogenic versus acetogenic substrates providing ME might alter the efficiency of EAA use. The objective was to evaluate milk yield and components in cattle fed diets with 2 levels of dietary starch and 2 levels of EAA supply. Lactating cattle (n = 96; 2.6 ± 1.3 lactations, 91 ± 22 DIM, 652 ± 83 kg BW) were blocked in 16 cow pens (n = 6) by parity, DIM, and BW and enrolled for 60 d as the first of 2 blocks in a randomized incomplete block experiment. Pens were fed a common diet during a 10-d covariate period and randomly assigned to 1 of 4 diets. Diets were fed with 2 levels of dietary starch (23% [LS] and 29% [HS] DM) and 2 levels of AAS (100% [100] and 105% [105] of requirements as described by CNCPS v7). Diets were isocaloric and balanced for AAS, with the 100% MP diets formulated for the optimum gAAS/Mcal ME and the 105% diets formulated at 5% excess AAS. Milk samples were collected weekly from 3 consecutive milking sessions. Results were analyzed using an ANOVA mixed model in SAS (v.9.4) and significance was declared at $P < 0.05$ and a tendency at $P < 0.1$. Cattle fed a HS diet consumed more DM (27.2 kg) than cattle fed a LS diet (24.1 kg; $P < 0.05$). Cattle fed HS105 made more ECM (45.0 kg ± 0.66 kg; $P < 0.01$) than cattle fed LS100 (40.9 ± 0.94 kg) and HS100 (40.8 ± 0.92 kg) and tended to make more than LS105 (42.8 ± 0.66 kg; $P = 0.09$). The HS105 treatment produced higher milk protein yield compared with other treatments (1.29 ± 0.02 kg; $P < 0.01$) whereas the HS100 (1.13 ± 0.03 kg) and LS105 (1.13 ± 0.02 kg) diets tended to yield more protein than LS100 (1.04 ± 0.03 kg; $P = 0.09$). Cattle fed the LS105 diet (14.5 ± 0.2 mg/dL) had higher MUN ($P < 0.05$) than LS100 (11.5 ± 0.3 mg/dL) and HS105 (11.6 ± 0.2 mg/dL) and tended to be higher than HS100 (12.3 ± 0.3 mg/dL). The data indicate when ME supply is more gluconeogenic, the efficiency of N and EAA use increases.

Key Words: amino acids, starch, nutritional models

W120 Performances and plasma energetic parameters in lactating dairy goats fed 3 levels of metabolizable methionine. P. Schmidely[1] and L. Bahloul*[2], [1]*Université Paris-Saclay, INRAE, AgroParisTech, UMR Modélisation Systémique Appliquée aux Ruminants*, *Paris, France*, [2]*Centre of Expertise and Research in Nutrition, Adisseo France S.A.S*, *Commentry, France*.

We characterized milk production and composition, and plasma parameters response to 3 levels of metabolizable methionine (MetDi) in 24 early-lactation Alpine goats (31 ± 3 DIM) genotyped for milk casein aS1. The 3 diets (14% CP, NEL = 1580 kcal/kg DM) supplemented with rumen-protected MetDi (RPM; isopropyl ester of 2-hydroxy-4-methylthio butanoic acid (HMBi, Metasmart, Adisseo) provided 90 (CTL), 100 (RPM100), and 110% (RPM110) of MetDi requirement based on cow requirement (INRA, 2007). The trial was conducted as a 3-groups randomized block design for 12 wk, with 2-wk covariate period. Goats were randomly allocated to their experimental TMR at the end of wk 2 according to their aS1 genotype (High vs Low), BW, DIM, milk yield (MY) and milk protein content (MPC). Milk and blood samples (before morning feeding and milking) were collected twice per wk and weekly, respectively. Data were analyzed using the MIXED procedure of SAS for repeated data with aS1 genotype and MetDi level as fixed factors. DMI did not differ between groups. Compared with Low aS1 goats, those with High genotype had higher MPC (+1.0 g/kg), higher milk fat content (+2 g/kg), with no difference in MY. Compared with CTL goats, RPM100 or RPM110 goats had similar MY (3.7, 3.7, 3.9 kg/d, respectively), similar fat, lactose and urea concentrations in milk. The RPM100 goats had significantly ($P < 0.07$) higher MPC (34.9 g/kg) than CTL (33.5 g/kg) goats. Compared with CTL goats, RPM100 or RPM110 goats had numerically higher protein yield: 123, 128 and 131 g/d ($P < 0.25$). MetDi supplementation × aS1 genotype interaction was not significant on MPC or protein yield. Compared with CTL goats, RPM100 or RPM110 goats had similar glucose, urea, BHB and insulin plasma concentration, but lower NEFA (0.19, 0.13, 0.16 μ*M*, $P < 0.05$). In conclusion, dietary MetDi recommendation in cows (2.5% metabolizable protein) can be used in goats whatever their aS1 genotype.

Key Words: metabolizable methionine, milk composition, dairy goats

W121 Effects of the supplementation with coated methionine on performance of dairy cows. L. R. Royo*, M. Puyalto, and J. J. Mallo, *Norel SA*, *Madrid, Spain*.

Effects of the supplementation with coated methionine on performance of dairy cows Two studies were carried out in commercial dairy farms with the aim to evaluate the effects of supplementing Methionine (Met) coated with palm stearin, on milk yield and composition. In the farm A, 160 Holstein cows (days in milk 171 ± 106, milk yield 36.3 ± 8.2 kg/d), and in the farm B, 93 Holstein cows (days in milk 181 ± 143, milk yield 38.9 ± 11.6 kg/d) were split in 2 homogeneous groups. In both farms, cows were exposed for 60 d to 2 treatments following a complete randomized design. Treatments consisted in a control (CT) group that received a basal diet (farm A: 20.1% CP, 30.5% NDF, 1.4 Mcal NE$_l$/kg; farm B: 21.6% CP, 36.5% NDF, 1.1 Mcal NE$_l$/kg; DM basis), and a Met supplemented group (BM) that received the same diets with less crude protein (CP; farm A: 18.4%; farm B: 21%) plus 40 g/d of coated Met (39% Met; providing 16 g of Met/d), commercialized as Bymet by Norel. In both farms the protein concentrate used were soy and rapeseed meal, the decrease in the CP content was set to show the economic benefits of reducing the CP. Cows were fed the ration twice daily. Milk yield was determined daily and milk composition (fat and protein) weekly. Data were summarized and analyzed using a mixed-effects model for repeated measures. In farm A, milk yield was affected ($P = 0.003$) by an interaction between treatment and week, BM cows produced greater amounts of milk during the whole study (36.8 kg/d), except in the wk 4, comparing with CT group (36.0 kg/d). In farm B, milk yield tended ($P = 0.110$) to be affected by the interaction between treatment and week, BM cows produced 39.8 kg/d and CT cows, 37.8 kg/d. Interactions between treatment and week were observed ($P = 0.01$ in farm A and B) for fat content, it was similar in both groups in farm A, but was higher in BM cows in farm B. Protein content was affected by an interaction between treatment and week ($P = 0.04$ in farm A and B), it was similar in both groups in farm A, but it tended to be lower in BM cows in farm B. It is concluded that Met coated with palm stearin can improve milk yield of cows fed a TMR with high levels of CP.

Key Words: methionine, milk, rumen

W122 Effect of calving on plasma amino acid concentration in dairy cows. M. E. Fetter*, D. M. Cunningham, F. Gambonini, T. L. Ott, and A. N. Hristov, *Department of Animal Science. The Pennsylvania State University*, *University Park, PA*.

Metabolic changes during the transition period in dairy cattle can cause decreased milk production and reproductive performance. Therefore, a better understanding of changes in plasma amino acid (AA) concentrations during the transition period will help optimize nutrition to improve welfare and production in dairy cows. The aim of this study was to assess changes in plasma amino acid (AA) profile after calving in Holstein cows. Blood samples for AA analysis were collected from the tail vein/artery of 9 multi- and 8 primiparous cows 3 wk (±7 d) before and at wk 1, 2, and 3 post-calving. Data were analyzed using the GLM procedure of SAS with sampling week, parity and parity × week interaction in the model. Concentration of the sum of essential AA (EAA) was similar between primi- and multiparous cows before calving but was on average 17.0% lower ($P = 0.009$) in multi- vs. primiparous cows postpartum. EAA concentration declined ($P < 0.001$) by 20.0% postpartum. The sum of non-essential AA (NEAA) increased linearly ($P < 0.001$) post-calving and was comparable between primi- and multiparous cows. Primiparous cows had 13.5%, 14.8% and 13.7% greater Leu ($P = 0.002$), Val ($P = 0.002$), and Ile ($P < 0.001$) concentrations, respectively, than multiparous cows. In addition, Val and Ile decreased 20.0% ($P = 0.003$) and 22.6% ($P < 0.001$) postpartum, respectively. Primiparous cows had 7.6% greater ($P = 0.04$) plasma His concentration than multiparous cows. Compared with prepartum, His decreased 13.1% ($P = 0.005$) postpartum in both primi- and multiparous cows. Met and Lys concentrations were not affected by parity ($P \geq 0.31$) but both AA decreased (22.0 and 24.5%, respectively; $P < 0.001$) after calving. Arg and Thr decreased ($P \leq 0.002$) 26.7% and 22.4% after calving but were not affected by parity. Overall, plasma concentration of EAA decreased after calving, indicating an increased demand for EAA around parturition. Lower postpartum plasma concentration of EAA in multiparous cows likely reflects an increased need for EAA to sustain greater milk production compared with primiparous cows.

Key Words: amino acid, calving, dairy cattle

W123 Methionine supplementation at low and adequate net energy supply in lactating dairy goats. S. Lemosquet[1], M. Boutinaud[1], A. Leduc[1], S. Binggeli[2], E. Chanat[1], and L. Bahloul*[3], [1]*INRAE, Agrocampus Ouest, PEGASE*, *Saint-Gilles, France*, [2]*Université Laval*, *Quebec, QC, Canada*, [3]*Center of Expertise and Research in Nutrition, Adisseo, France S.A.S*, *Commentry, France*.

The responses in milk yield (MY) and composition to methionine (Met) supply through the isopropyl ester of 2-hydroxy-4-methlythio butanoic acid (HMBi) at low (LE) and adequate levels (AE) of NE$_L$ (E) were inves-

Table 1 (Abstr. W123).

	Treatment					$P <$		
Item	LE	LEMet	AE	AEMet	SEM	E	Met	E×Met
MY, kg/d	3.06	2.93	3.03	3.24	0.07	0.07	0.42	0.03
MPC, g/kg	27.7	29.3	27.8	28.7	0.4	0.57	<0.01	0.29
MPY, g/kg	83.4	85.7	84.4	92.1	1.0	0.07	0.01	0.17
MFC, g/kg	38.0	39.4	39.9	41.0	0.8	0.02	0.08	0.82
MFY, g/d	115	116	121	131	2.6	<0.01	0.03	0.07
Lactose, g/kg	43.0	42.4	43.9	43.7	0.5	0.03	0.44	0.67
Lactose, g/j	131	126	135	141	6.0	0.09	0.94	0.33
Casein, g/kg	23.5	25.0	24.1	24.6	0.4	0.79	0.01	0.21
Casein, % MPC	81.0	80.5	82.8	81.7	0.8	<0.05	0.25	0.69
Met, μ*M*	22.6	27.5	26.5	25.8	1.6	0.47	0.17	0.07
AA group 2, μ*M*	136	129	169	126	9	0.10	<0.01	<0.05

tigated with a 2 × 2 factorial design on 48 multiparous Alpine goats (85 ± 15 DIM, 55 ± 6 kg), assigned to a randomized complete block design in 4 groups during 6 weeks. All goats received fixed amounts of hay (15.5 kg of DM per group) and of concentrates distributed individually with 0.24% of DM of HMBi added in LEMet and AEMet concentrates. A covariance-variance analysis was performed using the MIXED procedure SAS with CSN1S1 genotype, E, Met and E × Met interaction as fixed effects. The mean DMI of concentrates was 1.52 ± 0.12 kg/d/goat and DMI of hay were 13.4, 14.9, 14.3 and 14.2 kg/d in LE, LEMet, AE and AEMet groups, respectively. The NE_L density of LE vs. AE diets were 1.47 vs. 1.54 Mcal/kg DM, respectively with a same MP content (86 g/kg of DM; INRA, 2007). Results are shown in Table 1. Increasing Met supply increased MY at AE supply (E × Met). It increased milk true protein content (MPC) and yield (MPY) and casein content at both E levels. It tended to increase milk fat content (MFC) and increased its yield (MFY). It tended to increase the plasma concentration of Met and decreased the concentrations of AA from group 2 (Lys, Ile, Leu, Val) suggesting a higher mammary utilization of these EAA. Increasing E supply tended to increase MPY, and increased the proportion of casein in MPC. It also increased MFC, MFY, lactose content and tended to increase its yield. Supplying Met in lactating dairy goats had the same effects on milk composition than in cows but the increased MY is only observed at NE_L requirement (AE).

Key Words: isopropyl ester of 2-hydroxy-4-methlythio butanoic acid (HMBi), goat, energy

W124 Evaluating plasma methionine in response to feeding three rumen-protected methionine products.

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Rumen protected amino acids offer the opportunity for precise feeding of limiting amino acids to ruminants. Plasma methionine (Met) is a strong indicator of bioavailability of rumen protected Met products and is directly influenced by intestinal absorption of Met. This study examined the comparability of a new rumen protected Met product, KESSENT M, to 2 currently marketed products. Ten multiparous Holstein cows, 280 ± 73 DIM, were used in a replicated 3 × 3 Latin square design, with 7-d experimental periods. Treatments consisted of a control diet plus 12 g/d of either KESSENT M (Kemin Animal Nutrition and Health, Herentals, Belgium), Smartamine M (Adisseo Inc., Antony, France), or Mepron (Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany). Cows were fed ad libitum with 33% of their daily feed allotment provided every 8 h. Milking occurred at 4:30 a.m. and 3:30 p.m. daily with milk samples collected on d 5–7 of each period. During d 5–7 of each experimental period, blood samples were collected from jugular catheters at 2, 4, 6, and 8 h after the morning feeding. At the end of the experiment, samples were sent to Missouri Agriculture Experiment Station Chemical Laboratories for amino acid analysis by cation-exchange chromatography with an amino acid analyzer. There was no significant effect of treatment on DMI or production parameters. Plasma Met as a % of total amino acids minus Met was 1.5085, 1.5267, and 1.3622% for KESSENT M, Smartamine M, and Mepron, respectively. KESSENT M and Smartamine M were not found to be significantly different ($P = 0.3420$); however, KESSENT M and Mepron were significantly different ($P < 0.0001$), with KESSENT M yielding greater plasma Met Levels. There was a significant effect of time of sampling on plasma Met as a percentage of amino acids minus Met ($P = 0.002$), due to higher Met at 2 h (1.508%) than 4, 6, and 8 h (1.439, 1.447, and 1.469% respectively). Similarities in plasma Met levels between KESSENT M and Smartamine M treatments would suggest comparative bioavailabilities and bioavailability greater than that of Mepron.

Key Words: rumen-protected methionine, dairy cow

Author Index

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