

O006

Relationship between ruminal biohydrogenation products and milk fat depression

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Milk fat content is affected by physiological and environmental factors. Milk fat depression (MFD) is observed in ruminants fed diet rich in starch or unsaturated fat. Several works proposed that MFD was related to a modification of ruminal metabolism, favouring the increase of specific biohydrogenation (BH) intermediates with antilipogenic effects. This role was attributed to CLAt10c12 because it is the only intermediate that clearly inhibits milk fat synthesis. However, dose-response studies carried out in different situations suggested that other fatty acids (FA) might inhibit milk fat synthesis.

The aim of this work was to individuate FA able to explain MFD. At this aim, we proposed a multivariate approach to discriminate cows affected by MFD syndrome.

Individual milk FA profile of 40 cows in MFD (T) was compared with that of 45 control cows (C). The two groups were composed of Italian Holstein cows in mid-lactation. All animals were fed the same diet (21.4 Kg of DM: 5.4 Kg of alfalfa hay +6.3 Kg of corn silage +9.7 Kg of concentrate). The level of high moisture corn in the diet was higher than 20%. According to literature, cows were considered in MFD when milk fat content was lower than 2.4%. FA composition was determined by gas chromatograph, while CLA isomers profile was obtained by HPLC.

The stepwise discriminant analysis was first exploited to select FA that best discriminated the T and C status. Then, using the 8 selected FAs (CLAt10t12, CLAt9t11, CLAt7t9, CLAc11t13, C10:1c9, C18:0, C18:1t6-t8, C18:1c11), the discriminant analysis correctly assigned, after a bootstrap resampling procedure, 92% of to animals to one of the two status.

Results highlighted that MFD was related to a modification of BH pathway, which involved not only CLAt10c12, but also other CLA isomers. Moreover, further milk FA were able to discriminate MFD status like C18:0, C18:1t6-t8, C18:1c11 (involved in BH) and C10:1c9 (involved in mammary metabolism). To our knowledge, this is the first time that MFD is investigated by comparing cows fed the same diet. When diet conditions are similar between the groups, a multivariate approach is a useful tool to evaluate mutual relationship among different groups of FA. Interestingly, CLAt10c12 was not selected by discriminant analysis, although its role on

MFD is well known. Probably, the high correlation with other FAs masked its effect, reducing its discriminant power. On the contrary, CLAt discriminates more clearly the effect of MFD.

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O007

Effect of replacing soybean meal with rapeseed meal on productive performance and ruminal conditions of lactating dairy cows

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The objective of this study was to investigate the effects of different levels of rapeseed meal inclusion in high producing cows diets on feed intake, rumination, total tract digestibility, ruminal pH dynamics and productive performance. Eight multiparous Holstein cows were assigned to a 4 x 4 Latin square design, with 3 weeks adaptation and 1 week of collection. Diets differed on primary protein source, while other dietary components remained the same. Treatments were identified as: S (2.5% DM basis of soybean meal), CB (1.8% soybean and 1% rapeseed meal), CM (0.9% soybean and 2.25% rapeseed meal), and CA (3.5% rapeseed meal). Chemical composition of the diets was similar among treatments, and the CP amount was 14.6% on average. Dry matter intake, milk production and quality, and rumination time were measured daily. Ruminal pH was measured continuously at 10 min intervals. Fecal samples were collected at multiple time points and averaged daily. Diets and feces were analysed for aNDFom, ADF, ADL, undigestible NDF (uNDF₂₄₀) and potentially digestible NDF (pdNDF), in order to calculate total tract fiber digestibility. All data were statistically analyzed with a factorial arrangement of treatments using the PROC MIXED model in SAS.

Milk production was not different, while ECM resulted higher in CM treatment (34.63 kg/d in CM compared to 32.47, 33.73, and 33.63 kg/d in S, CB and CA respectively, $p < .05$). Dry matter intake was lower in S treatment compared to the others ($p < .05$), while rumination time was not different among treatments. Ruminal pH was higher in CM treatment compared to others (5.92 against 5.88, 5.84, and 5.71 in CM, S,

CB, and CA respectively, $p < .05$). pH dynamics were also different among treatment, since both the area at pH < 5.8 and < 5.5 were significantly lower ($p < .05$) in CM compared to other treatments. In particular, it resulted: 72.1, 107.2, 165.0, and 255.0 in CM, S, CB and CA, respectively, for pH < 5.8 ; 6.0, 15.0, 23.4, and 97.1 in CM, S, CB, and CA treatment, respectively, for pH < 5.5 . Total tract pdNDF digestibility decreased as the rapeseed inclusion increased, resulting in higher values for S treatment (61.25, 58.94, 56.63, and 54.32 in S, CB, CM, and CA respectively, $p < .01$). In conclusion, replacing soybean with rapeseed meal in diet for high producing cows led to an improved DMI, an increased ECM and protein amount, and to a more stable ruminal pH, with less time spent below sub-acidosis threshold.

0008

Study of the *in vivo* and *in vitro* variations of the enzymatic activity of bovine rumen fluid inocula

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Aim of the present work was to quantify the enzymatic activities (EAs) of rumen fluids (RFs) collected as inoculum in “*in vitro*” digestibility tests, in the attempt to assess the possible variations of RFs degradative capacity. A further objective was to evaluate the effects of the incubation of RFs on a standard substrate on enzyme expression by rumen microbes. In a first trial the activities of cellulase (C), amilase (A) and xylanase (X) were tested on 7 rumen fluid pools collected from two cows fed a hay-forage based diet in the interval June-September 2015. The EAs were tested using the radial enzyme diffusion method (RED) using substrate concentrations of 0.5%, 0.5% and 0.1% for C, A and X respectively. Wide variations of rumen fluid EA were observed with values (area of the halo surface; mm²) ranging from 295.75 to 621.70 for C, from 310.26 to 495.40 for A and from 95.00 to 723.17 for X. The differences observed between the rumen fluids examined were significant for all the enzymes ($p < .001$) The EAs decreased in August, probably in relationship to heat/dietary stress. In a second trial, 3 different pools of (RFs), collected from cows fed no (NC), medium (MC) or high concentrate (HC) diets, were incubated in triplicate “*in vitro*” on a standard substrate (lactating cows total mixed ration). The relative EA were tested at different intervals (h: 1, 2, 4, 8, 24 and 48) to evaluate the dynamic of C, A and X enzymes expression pattern by ruminal microbiota, and to

individuate the interval at which the different inoculums showed similar and/or maximum EAs. Initial EA were NC = 226.12; MC = 198.49 and HC = 282.93 mm² for A activity; NC = 250.32, MC = 365.67 and HC = 578.79 mm² for C; NC = 227.83; MC = 95.00; HC = 124.10 mm² for X. An increase of EA was observed for A from 4 to 8 hours, with maximum values of 321.71 for NC, 270.99 for MC and 304.27 mm² for HC. EA peaks were observed for C at 2 and 8 h. X activity showed a maximum value at 8 h for HC (208.67 mm²) and after 48 h for NC (343.5 mm²) and MC (288.07 mm²). Similar values of EA in the 3 pools (NC, MC and HC) were observed at 24 h for C and at 4 h for X. Concerning A, two (MC and HC) out of 3 pools showed similar EA values at 24 h. The RFs EA can vary widely across inoculums from the same animals. Incubations affects EA, generally inducing an increase in RFs coming from NC diet, and a decrease in RFs derived from HC diet, with similar values at variable intervals depending on the enzyme.

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0009

Effect of exhausted berries of myrtle on ruminal metabolism in dairy sheep

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Utilization of polyphenols-rich by-products has become very interesting for their potential use in animal nutrition as modulators of the end products of rumen fermentation. Exhausted myrtle berries (EMB) is a by-product of the maceration process of myrtle (*Myrtus communis* L.) berries to produce a commercial liqueur called *Mirto*. The objective of this study was to evaluate the effect of the dietary inclusion of EMB on ruminal metabolism in lactating ewes. Rumen liquor samples were collected at week 3, 7 and 11 of the trial from 18 Sarda dairy ewes randomly assigned to 3 dietary treatments; a control diet (CON), a diet supplemented with 50 g/d per head of EMB (EMB50), or a diet supplemented with 100 g/d per head of EMB (EMB100). Sampling was performed 2 hours after the morning feeding, using a stomach tube and an evacuation pump. The samples were analyzed for ammonia, volatile fatty acids (VFA), fatty acids (FA) profile and dimethylacetals (DMA). The methane production was estimated according to the equation of Moss et al (2000). Data were analyzed with the PROC MIXED procedure of SAS. The model included the