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Abbreviations: CTR, control; BCS, body condition score; DA, displaced abomasum; DIM, days in milk; LDG, lactodynamographic analysis; MY, milk yield; NIRS, near infrared system; PAMP, pathogen-associated molecular patterns; PDO, protected designation of origin; RFM, retained fetal membranes; RR, relative risk; SCC, somatic cell count; SCS, somatic cell score; TRT, treatment.

ABSTRACT

The effects of an immunomodulant feed supplement (OmniGen-AF®) were evaluated on cow health, composition and quality of milk produced for Parmigiano Reggiano cheese production. One hundred-ninety primiparous and multiparous Holstein and Crossbred dairy cows were randomly assigned to either a control (CTR, n=95) or a group fed 55g/h/d of the supplement (TRT, n=95), from dry off to 150 days in milk (DIM). Individual milk yield (MY) was recorded daily, and individual milk quality was analyzed monthly. Health events and involuntary culling were recorded. Daily feeding of the supplement did not produce any negative effect on composition and cheese making properties of milk used to produce Parmigiano Reggiano cheese. Casein content in the milk of primiparous TRT cows increased after 90 DIM, and milk cheese-making properties, like coagulation time (LDG, r') and aptitude (LDG, type) of cows fed the supplement were enhanced. TRT cows had fewer health related events (-21%) compared to CTR group, and multiparous TRT cows tended to have a lower somatic cell score (SCS) in the first 60 DIM than CTR (-0.6 pts). The incidence of clinical mastitis was observed to be lower in the TRT Holsteins cows than CTR (4 vs 11 cases). Involuntary culling was reduced in TRT group: supplemented cows had a lower culling rate within 60 DIM (1% TRT vs 7.4% CTR) and time of culling (DIM) occurred

later (102.6 and 57 DIM for TRT and CTR, respectively). These results suggest that cow health and milk quality can be improved through an appropriate nutritional strategy and the use of an immunomodulator supplement like OmniGen-AF®. This combined nutritional strategy could have important implications for strictly regulated products like Parmigiano Reggiano cheese, other PDO cheese or organic products. This strategy may provide a feeding option to better control animal health that is fundamental to satisfy the uprising consumer's expectations in minimizing the utilization of antimicrobials.

Keywords

Immunomodulator, somatic cell, milk cheese-making properties.

INTRODUCTION

Impact of diseases such as mastitis, ketosis, metritis and retained fetal membranes (RFM) on cow health, milk production and milk quality has been widely demonstrated. In particular, high somatic cell count (>300.000 cells/ml) is related to a lower production and lower milk quality for cheese production, due to the degradation of caseins operated by the proteases secreted by the somatic cells (Barbano et al., 1991; Gröhn et al., 2004; Larsen et al., 2004). The reduction in casein content affects cheese yield as well as the ripening aptitude of

cheese (Marino et al., 2005). This aspect is particularly important for cheeses like Parmigiano Reggiano and other Protected Designation of Origin (PDO) products that require a long period for ripening. The breakdown of caseins by somatic cell proteases is greatly reduced by the cooling temperature (Barbano et al., 1991), but Parmigiano Reggiano cheese, according to Consortium regulation (Consorzio del Formaggio Parmigiano Reggiano, 2011), is made using raw milk stored at a temperature not lower than 18 °C until delivery to the cheese factory. This step must occur within 4 hours from milking (Consorzio del Formaggio Parmigiano Reggiano, 2011), and cheese is consumed after 18-24 months of ripening. So good animal health and high quality milk is essential. Moreover, the entire production chain is strictly regulated: animal origin, feeding and herd management, additives and milk processing must be in compliance with the specific regulation, listed in the official rules of the Parmigiano Reggiano cheese production (Consorzio del Formaggio Parmigiano Reggiano, 2011). Rations are based on fresh forages and/or dry hay, while silages are not allowed (Mordenti et al., 2017). TMR is frequently prepared without adding water in the mixer wagon, in order to avoid unwanted fermentation (Fustini et al., 2017). Considering that forage:concentrate ratio in the diet must be 50:50 at least, good quality forages are necessary to meet the energy requirements of animals and milk quality (Fustini et al., 2017).

Because of this, dry matter intake needs to be improved. This requirement could be achieved by selecting highly digestible forages, thus presenting a low amount of indigestible NDF, or reducing the particle size of the diet (Bonfante et al., 2016; Palmonari et al., 2016, 2014).

Consumer's awareness and concerns about animal health and welfare is increasing and, likewise, the pressure to reduce the use of antimicrobials in farm animals, in particular in dairy cattle producing milk for PDO or organic dairy products (European Commission, 2011, 2007). The concern of antimicrobial resistance in humans is a reoccurring issue and in 2015 the European Commission launched an action plan against antimicrobial resistance recommending the importance of an appropriate use of antimicrobials in veterinary medicine (European Commission, 2011).

For all these reasons, in order to reduce disease prevalence in the herds it becomes essential to improve animal capability to resist different stressors including pathogens. The innate immune system represents the first barrier against incoming pathogens (Janeway and Medzhitov, 2002) and its efficiency is therefore essential for the maintenance of animal's health. For example, the activity of udder leucocyte populations plays a pivotal role determining the evolution of intramammary infections (Sordillo and Streicher, 2002). The impairment of the immune system is therefore hazardous for cow performance

and production and this is particularly evident during the peripartum period where cows experience a strong reduction of immune efficiency (Sordillo and Streicher, 2002). Thus, in addition to accurate herd management practices, it is important to supply the adequate nutrients or feed supplement, in order to support, together with milk production and pregnancy, the immune system (Ingvartsen and Moyes, 2013). Therefore, the development of efficient immunomodulatory strategies, safe and effective, is strongly recommended to support animal health and high quality productions (Sordillo and Streicher, 2002).

Some authors have recently reported the ability of a natural feed supplement (OmniGen-AF®) to increase leucocyte activity of supplemented dairy heifers, both for a long (Ryman et al., 2013) or a short period (Nace et al., 2014). OmniGen-AF® is a patented proprietary blend of ingredients demonstrated to support immune function in dairy cattle and other species (Phibro Animal Health Corporation, Quincy, IL, USA). Briefly, it contains a mixture of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, brewers dehydrated yeast, mineral oil, calcium carbonate, rice hulls, niacin supplement, biotin, d-calcium pantothenate, vitamin B-12 supplement, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, riboflavin-5-phosphate and folic acid. It has been demonstrated that OmniGen-AF® can activate the innate

immune system through the up-regulation of L-selectin (CD62L) mRNA expression (Nace et al., 2014; Ryman et al., 2013) and other neutrophil genes (Wang et al., 2009) compared to non-supplemented controls. These effects are potentially exerted by the additive through the interactions between PAMP (pathogen-associated molecular patterns), contained by yeasts and fungal organisms, and toll-like receptors of animals' gastrointestinal tract (Wang et al., 2007). Toll-like receptors are involved in microbe recognition and signal transduction activation to induce the expression of L-selectin and secretion of inflammatory chemokines (Iwasaki and Medzhitov, 2004). Ultimately, this sequence of events can contribute to the modulation of the adaptive immune response (Janeway and Medzhitov, 2002). As reported in these papers (Nace et al., 2014; Ryman et al., 2013), only heifers were used and no results are available about multiparous cows and involuntary culling. In addition, Ryman and co-authors (2013) evaluated only blood parameters while Nace et al. (2014) reported that their evaluation about health and milk quality was not sufficient due to the number of animals used in the project.

Therefore, the aim of our research was to evaluate the capability of this complementary feed supplement to reduce the incidence of pathologies, culling rate and somatic cell score, while maintaining milk production and quality, of primiparous

and multiparous cows in a farm that produced milk for Parmigiano Reggiano cheese.

METERIALS AND METHODS

Animals, housing and feeding

The experimental procedures were approved by the Scientific Ethical Committee for animal experimentation of Bologna University (PROT.CES N 78, 26/11/2013) in accordance with the EU Directive 2010/63/EU for animal experiments.

The experiment was conducted between November 2013 and September 2014, in a Parmigiano Reggiano dairy farm, located in the Po Valley region of Northern Italy. One hundred and ninety Holstein and crossbred cows (Italian Holstein x Montbeliarde) x Swedish Red Cattle) with expected calving dates between December 2013 and March 2014, were used in the study. Cows were paired by parity, breed, expected calving date, body condition score (BCS), somatic cell count (SCC) assessed one week before dry-off and previous milk yield of multiparous cows (Table 1).

Sixty days before expected calving, cows were randomly assigned to either control (CTR, n=95), or treatment group (TRT, n=95), where they remained until 150 DIM. Cows assigned to TRT were fed 55 g/cow per day of the supplement (OmniGen-AF®) for 210 days: 60 days of dry period plus 150 days of lactation. A single batch of supplement was used

throughout the entire trial. Analytical characteristics of the supplement resulted as following: ash 800.3, crude protein (N*6.25) 28.2, crude fibre 25.3, aNDF0m-NDF 45.1, crude fat 1.0, Ca 20.0, P 0.8, Na 4.2, Mg 3.8, g/kg as fed. The supplement was mixed with wheat grain and then added to the vertical mixer wagon equipped with scale (Storti, Vr, Italy), in order to guarantee an optimal distribution into the TMR.

From dry off to 150 DIM, cows moved through different boxes: far-off dry, close-up dry (-21 days to calving), fresh (1-10 DIM) and lactating (10-150 DIM) cows. Each box was designed to house two CTR and two TRT groups. In order to avoid any possible overstocking issues (Fustini et al., 2017), the experimental pens for far-off dry, close-up dry and lactating cows (10-150 DIM) were organized to house up to 55 animals. In particular, the four pens for lactating animals were designed to have 75cm width headlocks, and stalls with 260cm length and 130cm width. Pens (n= 4, 2 TRT and 2 CTR) for fresh cows (1-10 DIM) were organized to host 15 animals each. Pens for far-off (n = 4) and close-up dry (n = 4) cows were able to house up to 50 animals. Headlock width was 75cm, while the available space per cows was 10 m^2 for exercise area plus 8m^2 of resting area (straw bedded-pack).

All diets were fed to the CTR and TRT groups as total mixed rations twice a day and the composition of those diets is shown in Table 2. Diets were balanced using software based on

CNCPS model (DinamilkTM, v.5). All diets were formulated with feedstuffs and other ingredients as approved by Parmigiano Reggiano feeding regulation (Consorzio del Formaggio Parmigiano Reggiano, 2011). The supplement was fed at 55 g/cow per day and was added on-farm to each respective TRT dry and lactating cow group TMR. Cows BCS were recorded at dry-off, mid-dry, at calving and every 4 weeks thereafter to 150 DIM. BCS was assessed according to Edmonson method from 1 to 5 (Edmonson et al., 1989).

Cases of mastitis, ketosis, metritis, retained fetal membranes (RFM) and displaced abomasum (DA) were diagnosed by the on-sight veterinarian and recorded daily. In presence of clinical mastitis milk was sampled for microbiological analysis.

Definition of new clinical cases and calculation of incidence rate were made according to the method described by Kelton et al. (1998). Reasons and time of culling were recorded on all cows that left the trial.

Feed and milk samples

Feed and TMR samples were collected and analyzed monthly by Near Infrared Reflectance Spectroscopy (NIRS) (Brogna et al., 2018; Shenk and Westerhaus, 1985), using a NIRS instrument fitted with a spinning cup holder (NIRSystem 6500; Perstorp Analytical Inc., Silver Spring, MD).

Every three months, a TMR sample was analyzed by wet chemistry for the determination of: moisture, crude protein

(AOAC International, 1990, method 976.06 and 984.13), starch, aNDFom-NDF with addition of sodium sulfite, ADF and ADL (AOAC International, 1990; method 973.18), fat, and ash after 4 h combustion in a muffle furnace 550°C (Vulcan 3-550, Dentsply Neytech, Burlington, NJ). Supplement was analyzed, as well, by wet chemistry at the beginning of the trial, in order to determine its composition.

Feed orts from the CTR and TRT groups were recorded every two days and used to calculate daily average dry matter intake of each pen, by deducting the two days orts from the two days feed offered.

Lactating cows were milked twice a day and individual milk production was recorded daily by Afimilk system (Kibbutz Afikim, Israel) in the milking parlour.

In order to detect any possible effect of OmniGen-AF® on cheese production and quality, milk produced by the TRT and CTR groups was processed separately.

Milk samples were collected from all the multiparous cows one week prior to dry-off, to check the right balance among groups. Individual milk was then sampled at calving, and every 4 weeks thereafter from 30 to 150 DIM. Milk samples were immediately refrigerated at 4°C, and analyzed within 12 hours by a laboratory specialized in milk used for the manufacture of Parmigiano Reggiano cheese (Artest S.P.A., Modena, Italy). Every sample was analyzed for fat, direct casein, protein and

lactose percentage, somatic cell count (cell/ml), pH, titrable acidity (°SH), and coagulation properties. Milk components were measured by mid-infrared analysis (Biggs, 1978) with MilkoScan 6000 FT (Foss Eletric, Hillerød, Denmark). Precalibration procedures were performed according to International Dairy Federation Standards 141C:2000 (IDF, 2000), using total nitrogen for protein expression. SCC by flow cytometry with Combifoss apparatus, (Foss Eletric, Hillerød, Denmark) according to ISO13366-2:2006. Titrable acidity was determined by Soxhlet-Henkel method (Savini, 1946) and pH measurements using a potentiometric technique with Compact Titrator equipped with electrode P/N 53 64 (Crison Instruments, Barcelona, Spain). Coagulation properties, rennet coagulation time and aptitude, were evaluated were assessed by lactodynamographic analysis (LDG) with a Formagraph apparatus (Foss Eletric, Hillerød, Denmark) (McMahon and Brown, 1982) under isothermal conditions at 35 °C (Annibaldi et al., 1977). Rennet coagulation time (LDG, r') represents the minutes between the addition of rennet in the milk and the widening of the baselines in the graph generated from the analysis (Bittante et al., 2015; McMahon and Brown, 1982), while LDG type indicates the rennet coagulation aptitude. This classification qualifies milk samples according to their rennetability and takes into account clotting time (r'), curd-firming rate (k20) and curd firmness (a30). In Parmigiano

Reggiano cheese production, types A, B and C have good coagulation aptitude, D types are characterized by short r', short k20 and very high a30, while E and F poor clotting ability, with long and very long r' and k20 and weak a30 (Zannoni and Annibaldi, 1981).

In order to achieve normal distribution, somatic cell count data were transformed into somatic cell score (SCS) according to Shook and Schutz method (1994).

The Consortium of Parmigiano Reggiano evaluated and expertized all the cheese produced during the trial after 12 months of ripening, according to Parmigiano Reggiano regulation (Consorzio del Formaggio Parmigiano Reggiano, 2011).

Statistical analysis

The project design was a completely randomized model, with one dietary treatment (TRT) and one control diet (CTR).

Total milk production, disease incidence, culling rate and relative risk (RR) of culling before 60 DIM were evaluated on all cows included in the trial (n=190, 95 cows/ group). Forty-two cows out of 190 were excluded by the analysis of individual milk production and quality data: animals culled before the end of the trial (n=19), cows with missing values and animals with SCC in milk > 300,000 (n=23). Thus, a total of

148 cows (74 cow/group) were included in the analysis of individual milk data.

Normal distribution of the data was first tested by Shapiro Wilk test. Daily milk production, composition and quality data (fat, casein, protein, LDG time, SCS) were subjected to mixed model procedure with repeated measures with cow to serve as experimental unit, using the model reported in equation 1:

$$Y_{ijkl} = \mu + T_i + L_j + B_k + D_l + (T \times D)_{il} + \varepsilon_{ijkl} [1]$$

where Y_{ijkl} is the dependent variable; μ is the overall mean; T_i is the effect of treatment (Omnigen-AF or control); L_j is the effect of the number of lactation (j= 1 or > 2); B_k is the effect of breed (k= Holstein or crossbreed); D_l is the effect of days in milk (l= 11, 12…, 149, 150); $(T \times D)_{il}$ is the effect of treatment x days interaction and ε_{ijkl} is the random residual error. Treatment, lactation, breed, days in milk and treatment by day interaction were considered as fixed effects. Cow and pen were included as random effects, while terms of the repeated structure were days with cow as subject. Given that cows were group fed, individual dry matter intake was not available, therefore DMI data were not subjected to statistical analysis.

The same mixed model, excluding the time factor and the repetition over time structure, was used to analyze total individual milk production and days in milk at culling. Milk coagulation aptitude, health events and culling rates were tested by Fisher's exact test. For all tests, significant differences were

declared for P<0.05, highly significant for P<0.01 and trends for P< 0.10.

All data were processed using JMP® Pro (v. 12.0.1, 2015, SAS Institute, Cary, NC, USA).

RESULTS

Feed analysis and dry matter intake

Chemical composition of the rations of the two groups is reported in Table 3.

Individual dry matter intake, calculated from the average of pen feed consumptions, was (mean ± SD) 11.2 ± 0.9 and 11.5 ± 0.6 kg for CTR and TRT dry cows, respectively, and 22.3 ± 1.0 and 22.5 ± 0.8 kg for CTR and TRT lactating cows, respectively.

Milk production, composition and quality

Overall, no significant differences were detected in milk yield through 150 DIM (40.78 kg vs 41.97 kg, for CTR and TRT group, respectively) and no significant interactions were detected between parity, breed and dietary treatment (Table 4). Milk composition was maintained and it did not differ substantially between the groups (Table 5). Fat, protein and lactose content remained similar.

As shown in Figure 1, a significant treatment x time interaction was detected in casein content (g/kg) of milk produced by first calving heifers at 120 and 150 DIM (g/kg, TRT=27.5 vs

CTR=26.7, SEM 0.37, 0.P<0.05 and TRT=28.3 vs CTR=27.4, SEM 0.38, P<0.05). This increasing trend is appreciable at 90 DIM (g/kg, TRT=26.7 vs CTR=26.0, SEM 0.37, P=0.07), while no differences were observed for previous time points (30, 60 DIM). Similar situation was observed on clotting time (LDG, r'. Figure 2) for multiparous cows at 150 DIM (min., TRT=20.9 vs CTR= 23.1, SEM 0.96, P<0.05).

Milk coagulation aptitude was improved in the TRT group, as reported in Table 6: the number of milk samples with defect clotting aptitude (LDG type E and F) was significantly lower (P<0.01) in milk collected from the TRT cows compared to the CTR (111 vs 146, respectively). Opposite situation was observed in milk samples with suboptimal aptitude (LDG type D), which were less in CTR group (47 vs 75 for CTR and TRT group, respectively; P<0.01).

Due to the exclusion of unhealthy or culled cows, also the SCS were similar among groups, with a slight improvement appreciable in the TRT group.

Cows fed the TRT diet had numerically lower somatic cell scores (SCS) than CTR cows throughout the trial (TRT, SCS=2.68; CTR, SCS=2.92) and tended (P<0.10) to be on average 0.6 points lower for the multiparous TRT cows during the first 60 DIM (Figure 3).

Throughout the trial, cows fed the TRT diet had a significant (P<0.05) reduction of the milk samples with SCC over 300,000

cells/ml compared to the CTR: 123 samples (27.7 %), TRT vs 147 samples (33.1 %), CTR.

At the Official expertisation performed by the Consortium after 12 months of ripening, 98% of cheese produced during the trial was marked as first quality cheese.

BCS, health events and culling rates

The CTR and TRT groups were balanced for BCS at dry off and no differences were detected between the groups over the length of the study.

Incidence of infectious and metabolic diseases for the Holstein and Crossbred cows, cows culled included, are reported in Table 7. Holstein TRT cows had fewer cases of clinical mastitis than Holstein cows in CTR group (4 cases vs 11 cases; $P<0.05$). No difference was detected among Crossbred cows for mastitis. Incidence rates of the other recorded diseases were not different between the CTR and TRT cows. The majority of these cases were caused by *Streptococcus uberis* (5 cases in TRT group and 7 cases in CTR group) while a minor part of that was caused by *Staphyloccocus aureus* (3 cases in TRT and 4 cases in CRT group) and *Escherichia coli*, (1 cases in TRT group and 2 cases in CRT group) with no difference between the groups in the causing agent. In 3 cases no specific pathogens were detected due to milk samples contamination. Among the animals with mastitis, 6 were culled due to the

consequences of the disease: 4 in control group and 2 in the treated one.

A discrete amount of animals (19) was culled for health issues before 150 DIM (CTR, n=12; TRT, n=7). Culling rate at 60 DIM was significantly lower ($P<0.05$) in TRT group (1.0%) compared to CTR (7.4%). Holsteins (n=8) were the only breed culled before 60 DIM. Considering all breeds, CTR cows were culled earlier in milk than TRT (57.3 and 102.6 DIM for CTR and TRT group, respectively; $P<0.05$) and the risk of being culled before 60 days of lactation was 7 times higher (RR = 7, $P<0.05$) for cows fed the CTR diet than those on the TRT diets.

DISCUSSION

The impact of disease and high SCC on milk production has been widely demonstrated (Fourichon et al., 1999). A recent study (Hand et al., 2012) evaluated milk loss relative to SCC and reported that as SCC increased from 200 to 2000 ($x10^3$ cell/ml), the milk production decrease ranged from 0.35 to 4.7 kg/day. Other authors (Gröhn et al., 2004) reported that milk yield starts decreasing several weeks before mastitis becomes evident to reach the minimum only after clinical diagnosis.

In our study, the healthier status of cows in the TRT group supports the lower culling rate in this group. Our results agree with those reported in a study using the same feed supplement in dairy heifers (Eubanks et al., 2012). The authors compared

milk production, SCC and the expression of genes associated with the function of circulating immune cells of 83 heifers during transition. In this study, after two weeks of lactation, supplemented heifers produced on average 4 kg/day more milk than control, had a lower prevalence on mastitis (4 vs 13 %) and lower SCC (180 vs 711 $x10^3$ cell/ml). However, an increase in milk production is not always reported with the use of this supplement (Nace et al., 2014). The reduction of somatic cells in milk observed in this experiment could also explain the difference in casein content between TRT and CTR primiparous cows (Figure 1), and the improved coagulation properties of milk from the TRT group that had shorter clotting time (LDG, r', Figure 2) and fewer samples with bad (E and F) clotting aptitude (30.0 vs 39.9 %, P<0.01, Table 6). Type E and F milk, indeed, are characterized by long coagulation time and scarce reactivity to rennet, typical of cows with high SCC and mammary gland disorders (Bittante et al., 2012).

On the contrary, type D milk that resulted to be higher in TRT group, is characterized by quick coagulation process (short r', and k20) and high firmness of the curd (a30>50mm) that are characteristic of milk with high casein content and low titratable acidity (Bittante et al., 2012). This aspect is particularly important when milk is processed for PDO cheese production, like Parmigiano Reggiano (Bittante et al., 2011). Coagulation properties of milk, indeed, significantly influence

cheese yield and composition (Malacarne et al., 2006; Ng-Kwai-Hang et al., 1989). These characteristics of milk are strictly related to its casein content and genotype, which in turn are influenced by breed, health and nutrition of cows (Bittante et al., 2012; Malacarne et al., 2006). Formaggioni et al. (2015), recently, proposed a new predictive equation for cheese yield based on casein content as key factor and then on milk fat.

On the contrary, somatic cells have detrimental effects on these cheese making properties and final cheese yield (Summer et al., 2015) because of the enzymatic degradation of proteins operated by proteases (Larsen et al., 2004). Actually, a significant reduction in Parmigiano Reggiano cheese yield was reported by Summer et al. (2015) when milk somatic cells are above 300,000 per milliliter.

As expected, fat, protein and lactose content of milk did not differ between the groups (table 5), confirming the existing literature that reports even in heat stressed cows, no influence of this supplement on milk composition (Hall et al., 2014; Leiva et al., 2017)

Cheese evaluation performed after 12 months of ripening showed results comparable to the average of the whole Parmigiano Reggiano production, in fact, unpublished data from Consortium refers that only the 2% of Parmigiano Reggiano is not marked as first quality cheese. Therefore, considering the results obtained in this experiment, we can

speculate that the supplement does not negatively interfere with the final evaluation of cheese performed by Consortium experts.

In regards to the animal resistance to pathologies, the immune system plays a key role. Its functionality and effectiveness is partially related to adequate supply of macro and micronutrients that exert their activity through their antioxidant capabilities (Sordillo, 2016). During the dry and transition period, a nutritional imbalance like impaired energy and availability (Formigoni et al., 2003) and the lack of certain vitamins and minerals can impair host immune defense mechanisms and will lead to an associated higher risk of diseases (Formigoni et al., 2011; Ingvartsen and Moyes, 2013; Sordillo, 2016; Zhao et al., 2015). Although the direct study of the activity of immune cells could give more precise information, the evaluation of the incidence of pathologies is a useful approach to understand immune competence of animals (Sordillo, 2016).

In the study presented here (Table 7), cows in the TRT group reported a lower number of total clinical cases, and in particular, Holstein TRT cows had fewer clinical cases of mastitis than Holstein controls (11 and 4 in CTR and TRT group, respectively; $P<0.05$). In contrast, no significant difference was found between groups among Crossbred animals. Similar results were reported by others investigating

OmniGen-AF® supplementation and incidence of health issues with reports of fewer cases of diseases and a tendency for less new mastitis in supplemented cows (Nace et al., 2014). The efficiency of this supplement to reduce new mammary infections was first reported by Rowson et al. (2011). In this study the authors described a significant reduction in pathogen DNA concentration in mammary glands of mice fed with the supplement for two weeks prior to infection with bovine-origin bacterial isolates. In addition, they reported increased mRNA expression of myeloperoxidase and major histocompatibility complex in mice fed the supplement indicating a more robust inflammatory response and antigen presentation.

In our study, OmniGen-AF® supplementation to dry and lactating cows reduced culling rates within 60 DIM (1 vs 7.4 %, $P<0.05$) and extended cows production life. Interestingly, this is the first study to report results about improved involuntary culling with OmniGen-AF® supplementation. It is important to note that all culled animals were Holstein cows while no Crossbred cow was culled before the end of the trial (8 vs 0, $P<0.05$). Our results agree with Heins data (Heins et al., 2006), who reported higher survival rates of Crossbreds compared to pure Holsteins, as well as better reproductive performance. These data suggest a higher disease susceptibility of the Holstein breed compared to Crossbreds and our results

suggest a better effectiveness of OmniGen-AF® supplementation to Holstein animals.

Stronger evidence of the activity of OmniGen-AF® in optimizing the immune system could be gained by further research that combines transition cow performance with immunity or stress challenge and direct evaluation of metabolic status and protein markers or immune function.

CONCLUSIONS

In our study, the use OmniGen-AF® in dairy cows feeding, from dry off to 150 DIM, improved health status of animals by reducing incidence of disease and culling rate within 60 DIM, without negatively influencing milk composition and cheese making properties.

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Table 1. Animal features of control (CTR) and treatment (TRT) group, evaluated at the beginning of the trial (average ± standard deviation).

	Group	
Measure	CTR	TRT[1]
Animals, n	95	95
Primiparous	32	31
Multiparous	63	64
Holstein	61	63
Crossbred[2]	34	32
Parity, n	2.1 ± 1.15	2.2 ± 1.18
BCS[3], points	3.5 ± 0.4	3.4 ± 0.3
Milk yield, kg/day[4]	32.8 ± 6.6	33.1 ± 5.3
SCS[5], points	4.7 ± 2.3	4.5 ± 2.0

[1] TRT animals were supplemented with 55 g/day per cow of OmniGen-AF®.

[2] Crossbred: (Italian Holstein x Montbeliarde) x Swedish Red Cattle.

[3]BCS: Body Condition Score (Edmonson et al., 1989).

[4]Previous lactation averaged milk yield of multiparous cows.

[5] SCS: Somatic Cell Score.

Table 2. Ingredients of lactating and dry cows rations. (Treated group (TRT) was supplemented with 55 g/day per cow of OmniGen-AF®).

	Stage of lactation		
Ingredient, kg (as fed)	Lactating	Far-off dry	Close up dry
Wheat straw	--	3	3
Grass hay (1st cut)	2.5	5	5
Alfalfa hay	7.5	--	--
Grass hay	--	At pleasure	At pleasure
Barley meal, fine	2	--	--
Wheat meal, fine	1.5	1.5	1.5
Corn	6	--	--
Corn meal	3	--	--
PAM[1]	3.5	--	--
Dry mix[2]	--	1	3
Enerfeed 4[3]	1	--	--
Nectar[4]	1	--	--
Water	--	3	--

[1] supplementary feeding for lactating cows (g/kg a.f): C.P 330, C.F 115, crude fat 38, ash 97, Na 6. Ingredients: soybean meal, sunflower meal, wheat bran, soy hulls, soybean whole, Ca carbonate, NaCl, Na-bicarb, MgOX, vit. premix (VitA = 120000 IU/kg, VitD = 8000 IU/kg, VitE = 400 mg/kg), micro minerals (Zn = 500 ppm, Mn = 500 ppm, Cu = 75 ppm, I = 45 ppm, Se = 5 ppm).

[2] supplementary feeding for dry cows (g/kg a.f): C.P 303, crude fat 37, C.F 0, ash 105. Ingredients: sunflower meal, soybean meal, wheat bran, corn gluten feed, soybean whole, barley, cane molasses, Ca carbonate, NaCl, Na-bicarb, MgOX, vit. premix (VitA = 30000 IU/kg, VitD = 6000 IU/kg, VitE = 1000 mg/kg, VitB1 = 15 mg/kg, VitB2 = 15 mg/kg, VITB6 = 11 mg/kg, VITB12 = 0.04 mg/kg), micro minerals (Zn = 1500 ppm, Mn = 1500 ppm, Cu = 250 ppm, I = 100 ppm, Se = 15 ppm).

[3] molasses (g/kg a.f): moisture 280 g/kg, sugars 240, C.P 75, crude fat 2, C.F 1, ash 90, Na 9.

[4] supplementary feeding for lactating cows (g/kg a.f): C.P 310, C.F 50, crude fat 103, ash 65, Na 2.6. Ingredients: soybean whole, soybean meal, glicerine, lineseed, Ca carbonate, NaCl, Na-bicarb, MgOX, vit. premix

(VitA = 100000 IU/kg, VitD = 4000 IU/kg, VitE = 250 mg/kg, VitB1 = 10 mg/kg, VitB2 = 12 mg/kg, VITB6 = 18 mg/kg, VITB12 = 0.02 mg/kg, Nicot. Ac. = 6000 mg/kg, Coline = 2000 mg/kg, L-Carn. = 280 mg/kg), micro minerals (Zn = 150 ppm, Mn = 150 ppm, Cu = 25 ppm, I = 35 ppm, Se = 5 ppm).

Table 3. Averaged chemical composition (g/kg dry matter, DM) of lactating and dry cow rations fed to control (CTR) or supplemented (55 g/d/h of OmniGen-AF®) cows (TRT).

	CTR			TRT		
Item, g/kg DM	Far-off dry	Close-up	Lactating	Far-off dry	Close-up	Lactating
Samples, n	11	11	11	11	11	11
Dry matter, g/kg	721	751	885	695	777	892
Crude Protein,	109	143	172	95	150	172
Starch	24	197	291	33	192	281
aNDFom-NDF[1]	610	402	269	66	397	267
ADF	358	204	205	373	204	211
ADL	66	40	35	67	38	37
E.E.	16	25	26	16	26	27
Ash	95	87	89	95	93	93

[1]aNDFom-NDF: alpha-amylase treated NDF, ash corrected.

Table 4. Least square means of daily individual milk production (kg) and number of cows (n) of supplemented (TRT, n = 74) and control (CTR, n = 74) cows divided for parity and breed from 11 to 150 DIM.

	Cows, n		Milk, kg			
Parity	CTR	TRT[1]	CTR	TRT[1]	SEM[2]	P-VALUE
Primiparous						
Holstein	23	21	35.2	36.2	0.89	0.63
Crossbred[3]	8	8	33.6	34.6	0.89	0.55
Multiparous						
Holstein	21	29	45.5	46.5	0.89	0.71
Crossbred[3]	22	16	43.9	44.9	0.89	0.53

[1] TRT animals were supplemented with 55 g/day per cow of OmniGen-AF®.

[2] SEM: standard error of the mean

[3]Crossbred: (Italian Holstein x Montbeliarde) x Swedish Red Cattle.

Table 5. Least square means of composition and characteristics of milk produced by supplemented (TRT, n = 74) and control (CTR, n = 74) groups, sampled at calving, and every 4 weeks thereafter from 30 to 150 DIM.

	Group			
Item	CTR	TRT[1]	SEM[2]	P-VALUE
Composition, g/kg				
Fat	28	28	0.09	0.97
Protein	34	34	0.03	0.91
Casein	26	26	0.02	0.53
Lactose	49	50	0.02	0.48
Titrable acidity, °SH	3.8	3.8	0.04	0.85
LDG[3], r', min	19.6	18.5	0.69	0.74
SCS[4], points	2.8	2.5	0.26	0.63

[1]TRT animals were supplemented with 55 g/day per cow of OmniGen-AF®.

[2]SEM: standard error of the mean

[3]LDG: milk clotting time evaluated through lactodynamographic analysis.

[4] SCS: somatic cell score.

Table 6. Number (n) and percentage (%) of milk samples with good (A, B, C), suboptimal (D) and defect (E, F) coagulation aptitude, evaluated through lactodynamographic analysis (LDG). Individual milk was sampled at calving, and every 4 weeks thereafter from 30 to 150 DIM in both supplemented (TRT, n=74) and control (CTR, n=74) groups.

LDG, type	Group				Chi-square, X^2	P-value
	CTR		TRT[1]			
	n	%	n	%		
Total analysis	366		370			
A, B	173	47	184	50	0.45	0.50
C	0	0	0	0	-	-
D	47	13	75	20	7.34	< 0.01
E, F	146	40	111	30	7.92	< 0.01

[1] TRT animals were supplemented with 55 g/day per cow of OmniGen-AF®.

Table 7. Number of clinical cases recorded in control (CTR, n=95) and supplemented (TRT, n=95) cows, from calving to 150 DIM or culling, and percentage of animals culled within 60 DIM.

	Group			
Measure	CTR	TRT[1]	SEM[2]	P-value
Holstein				
Animals, n	61	63	1.81	0.51
Ketosis	1	1	0.03	0.97
Clinical mastitis	11	4	0.42	< 0.05
Metritis	18	17	0.23	0.63
RFM[3]	8	7	0.11	0.74
Culled, %	7.4	1.0	0.25	< 0.05
Crossbred				
Animals, n	34	32	1.37	0.76
Ketosis	3	2	0.12	0.83
Clinical mastitis	5	5	0.34	0.96
Metritis	5	8	0.32	0.47
RFM[3]	3	3	0.28	0.90
Culled, %	0	0	0	-

[1] TRT animals were supplemented with 55 g/day per cow of OmniGen-AF®

[2] SEM: standard error of the mean

[3]RFM: retained fetal membranes.

Figure captions

Figure 1. Effect of feed supplementation during dry and lactation period on casein content (g/kg) of milk produced by primiparous supplemented (TRT) and control (CTR) cows from 11 to 150 DIM, expressed as average value (data point) and standard error (error bars). Casein content differed significantly at DIM 120 and 150.

* = $P < 0.05$.

Figure 2. Effect of feed supplementation during dry and lactation period on clotting time (r', minutes) of milk produced by multiparous supplemented (TRT) and control (CTR) cows from 11 to 150 DIM, evaluated by lactodynamographic analysis (LDG), expressed as average value (data point) and standard error (error bars). Clotting time was significantly shorter for TRT multiparous cows at DIM 150.

* = $P < 0.05$.

Figure 3. Effect of feed supplementation during dry and lactation period on somatic cell score (SCS) of multiparous supplemented (TRT) and control (CTR) cows from 11 to 150 DIM expressed as average value (data point) and standard error (error bars). SCS tended ($P < 0.1$) to be lower in multiparous TRT cows at 30 and 60 DIM.