



Immunomodulant feed supplement to support dairy cows health and milk quality evaluated in Parmigiano Reggiano cheese production

L.M.E. Mammi^{a,*}, A. Palmonari^a, M. Fustini^a, D. Cavallini^a, G. Canestrari^a, J.D. Chapman^b, D.J. McLean^b, A. Formigoni^a

^a Department of Veterinary Medical Sciences, Università di Bologna, Bologna, Italy

^b Phibro Animal Health Corporation, Quincy, IL 62305, USA

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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 55 g/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.

1. Introduction

Impact of diseases such as mastitis, ketosis, metritis and retained fetal membranes (RFM) on cow health, milk production and milk

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

* Corresponding author at: Department of Veterinary Medicine, Università di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Italy.

E-mail address: ludovica.mammi@unibo.it (L.M.E. Mammi).

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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 h 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 (Ingvarsen 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 et al. (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.

2. Materials and methods

2.1. 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 $\times$ Montbeliarde) $\times$ Swedish Red Cattle) with expected calving dates between December 2013 and March 2014, were used in the study.

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