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Does the dry cow treatment with Monensin controlled release capsule impact Parmigiano Reggiano cheese production?

Mammi

This study aimed to investigate the effects of a recent preventative treatment for ketosis in dairy cows on Parmigiano Reggiano cheese production and quality.

Based on the use of unpasteurized milk and the unique characteristics of this cheese, the sustained release formulation of this treatment raised some concerns from the Italian dairy industry on potential effects in cheese making processes. This study suggests that the monensin intraruminal device does not negatively affect cheese making process, cheese composition or sensory characteristics.

EFFECT OF MONENSIN ON PARMIGIANO REGGIANO

Does the dry cow treatment with Monensin controlled release capsule impact Parmigiano Reggiano cheese production?

L.M.E. Mammi[*1], L. Grazia[†], A. Palmonari[*], G. Canestrari[*], A. Mordenti[*], M. Vecchi[‡], F. Archilei[‡], A. Formigoni[*]

[*] Department of Veterinary Medical Sciences, and

[†] Department of Agricultural and Food Sciences, University of Bologna, Ozzano Emilia, (Bo) Italy.

[‡] Elanco Italia SpA, Sesto Fiorentino (FI), Italy

[1] Corresponding author: Ludovica Mammi, via Tolara di Sopra 50, 40064, Ozzano Emilia (BO) Italy, Phone +39 051 2097395, e-mail ludovica.mammi@unibo.it

ABSTRACT

In this study we investigated the effects of monensin controlled-release capsule (**CRC**) (Kexxtone, Eli Lilly and Company Ltd, United Kingdom) preventative ketosis treatment on traditional cheese making process as well as the final characteristics of Parmigiano Reggiano (**PR**) cheese.

The use of this prevention product to reduce the incidence of ketosis in transition dairy cows was approved by the European Medicines Agency in 2013. There are no previous experiences available concerning the effects of this treatment on prolonged ripening cheeses production such as PR. In PR cheese production, feed, feed additives and cow treatments are strictly regulated in order to avoid any possible interference with traditional manufacturing processes.

For these reasons, in one farm where all milk is used for PR cheese production, monensin CRC was administered to 33 cows, 21 days before calving in the monensin treated group (**TRT**), while untreated cows with similar breed and parity characteristics constituted the control group (**CTR**).

For 20 weeks, milk obtained from each group and whey starter were separately managed and transported in the cheese factory, where 2 cheese wheels per group were produced daily, making 552 PR cheese wheels in total. Morning bulk tank milk composition, cheesemaking properties and whey starter fermentation activities were analyzed twice a week. Every aspect of the cheesmaking process was recorded and the resulting cheese was evaluated after 36 hours, 6, 12 and 18 months from production for yield, texture defects, composition and fatty acids profile. Milk from the two groups differed for somatic cell content (TRT 3.04 *vs* CTR 4.06, Somatic Cell Score p.ts), total bacterial count (TRT 4.08 *vs* CTR 6.08, *1000 UFC/ml), titratable acidity (TRT 3.66 *vs* CTR 3.72, °SH/50ml) and casein content percentage (TRT 2.4 vs CTR 2.5, %). Whey starter parameters were comparable between the two groups. Final cheese composition and organoleptic profile were not influenced by the treatment except for C18:1 content being enhanced (TRT 22.8 vs CTR 20.8, % of fatty acids). Percentage of defected ripened cheese was significantly lower in the treated group, both at x-ray evaluation performed at 6 months (TRT 6.2 vs CTR 12.3, %) and at the Consortium inspection, performed at 12 months of ripening (TRT 1.5 vs CTR 6.5, %). On the other hand, average cheese yield at 18 months of ripening was partially reduced (TRT 7.5 vs CTR 7.7, %).

Overall in this study, the use of monesin CRC had no negative effect on the cheesemaking process, prolonged ripening cheese characteristics, milk composition or whey starter quality.

Key words

Monensin, milk quality, Parmigiano Reggiano, cheese quality

INTRODUCTION

Ketosis is one of the most important diseases in modern herds due to its high incidence and its deep impact on cow health and performance. Recent studies reported that subclinical ketosis (SCK) incidence, within the first 16 days of lactation, varies from 22 to 43% in European and American herds respectively (McArt et al., 2012; Suthar et al., 2013). Cows affected by subclinical or clinical ketosis have a higher risk of developing pathologies such as displaced abomasum and metritis as well as risk of culling as a consequence of health problems (Duffield et al., 2009; McArt et al., 2012; Suthar et al., 2013). Reproductive performance of these animals is often impaired and milk production reduced (McArt et al., 2015) together with changed composition. Indeed, ketosis reduces the protein content of milk on first DHIA test day (Vanholder et al., 2015) and may consequently impair its cheese making properties.

In 2013, the European Medicines Agency (EMA) approved a new treatment for prevention of ketosis in dairy cows: a monensin controlled release capsule (**CRC**) (Kexxtone, Eli Lilly and Company Ltd, United Kingdom).

Monensin is a carboxylic polyether ionophore commonly used as a feed additive in ruminants to alter rumen fermentation in order to improve energy efficiency (Russell and Strobel, 1989). Its effects on energy metabolism are well known and widely described both in beef and dairy cattle (Goodrich et al., 1984; Ipharraguerre and Clark, 2003; Duffield et al., 2012). Monensin has a selective action on rumen microbes: it alters ion exchange through the inner and outer membranes of microbial cells. In this way it reduces the prevalence of protozoa and gram positive population and promotes gram negative proliferation, that is mainly responsible for propionate production (Russell and Strobel, 1989). As a consequence, the ratio between acetate and propionate changes in favor of propionate, thereby improving energy metabolism of cows (Russell and Strobel, 1989).

Monensin administration as a feed additive is not allowed in Europe; consequently, its introduction in 2013 as a ketosis prevention product created a concern in the Italian dairy industry that there may be negative effects on the quality of cheese following production.

In recent years, numerous studies have investigated the effects of monensin administration on animal metabolism and performance and regardless of whether or not it is administered as a feed additive or controlled release capsule, the beneficial effects have included reduced NEFA and BHBA plasma concentration, increased propionate production in the rumen and decreased incidence of clinical and subclinical ketosis (Duffield et al., 1998). On the other hand, only a few studies have explored the effects on milk quality and these have shown contrasting results. No studies, to our knowledge, have assessed the impact of monensin on cheese quality. Mullins (Mullins et al., 2012) did not find any changes in milk production and composition in monensin treated cows, while other authors found a significant reduction in milk fat and protein content percentage (Odongo et al., 2007; Duffield et al., 2012).

Parmigiano Reggiano cheese is traditionally made with raw, unpasteurized and partially skimmed milk. To produce this kind of cheese, feedstuff, management and milk processing must be in compliance with Parmigiano Reggiano regulations (Consorzio del Formaggio Parmigiano Reggiano, 2011) by virtue of the Ministerial Decree in force since October 1st 2011, that implement the European regulation for PDO production (Council Regulation, n 510/2006). Cows are fed without silages and therefore, in order to maintain milk production and composition and to avoid ruminal disorders, a proper inclusion of high quality hays in the ration is always needed (Fustini et al., 2017).

In this specific manufacturing process, milk composition and environmental wild microflora are extremely important (Mordenti et al., 2017). Indeed, microbial population of whey starter is fundamental for the quality and the maturation process of the cheese (Coloretti et al., 2016). Considering its antimicrobial activity, some have suggested that the administration of monensin might potentially impair cheese composition and quality. Therefore, the main purpose of our study was to evaluate the effect of a mass treatment of dry cows with monensin CRC on Parmigiano Reggiano cheese production.

MATERIALS AND METHODS

Animals, Feeding, Management conditions and Treatment

In the European Union, monensin use is restricted only to cows considered to be at high risk for ketosis. Consequently, the experimental design used in this study resulted in a more extreme scenario in which mass use of monensin controlled release capsule (**CRC**) was required. This is typical of the summer heat stress period, when all cows are considered to be at high risk of ketosis. The treatment, monensin CRC (Kexxtone, Elanco Animal Health, Eli Lilly and Co. Ltd, UK), contained 32.4 g of monensin released continuously in the rumen throughout 95 days, at a daily dose of 335 mg (EMA, 2013).

Cows involved in the study were divided into two groups, Treated (**TRT**) and Control (**CTR**), and housed in two comparable, dedicated pens, with a straw bedded resting area with cubicles. 33 cows received the treatment 21 days before their expected calving date and gradually entered the TRT study group around 10 DIM, once milk became eligible for processing, according to Parmigiano Reggiano regulations (Consorzio del Formaggio Parmigiano Reggiano, 2011).

The percentage of cows in the TRT group within 95 days from treatment administration increased from 50% at the beginning of the trial to a maximum of 80% during the 7th week of study. In the last 5 weeks, the percentage of treated cows gradually decreased until 0. The percentage of cows under treatment throughout the trial is shown in Figure 1.

All health problems were recorded as well as pharmaceutical treatments. Milk from cows treated with antimicrobials during the trial was not used for cheese manufacturing in the experimental groups for a period equal to double the standard withdrawal time in order to avoid any possible interference of the molecule on milk and whey starter quality. As soon as a cow exited the TRT group, new untreated cows entered, in order to maintain a minimum of

29-30 cows per group and to have at least 1000-1100 kg of milk/day/group, sufficient to produce 2 cheese wheels a day from each group.

During the experiment, both groups received the same TMR, delivered twice a day. The ration was formulated according to Parmigiano Reggiano feeding rules (Consorzio del Formaggio Parmigiano Reggiano, 2011). Samples of TMR were collected monthly and analyzed using NIR equipment for moisture, crude protein, starch, aNDFom with addition of sodium sulfite (Mertens, 2002), ADF and ADL, fat, and ash after 4 h combustion in a muffle furnace 550°C (Vulcan 3-550, Dentsply Neytech, Burlington, NJ). Ingredients and chemical composition of the diet are shown in Table 1.

Milking and cheese production

Cows of both groups were milked separately, twice a day and milk was stored in separated tanks. Milk and whey starter obtained from the two experimental groups were maintained separately from each other and from the rest of the herd during every phase of the cheese making process using two different copper vats for the cooking procedure and two different comparable tanks for the storage of whey starter.

Each day, 2 cheese wheels per group were produced and marked following Parmigiano Reggiano cheese production standards (Consorzio del Formaggio Parmigiano Reggiano, 2011). Cheese wheels of both groups were stored together in the same traditional ripening rooms for 18 months.

Milk, whey starter and cheese analysis

Every day the amount of milk produced and delivered to the cheese factory by the two groups was recorded. Morning bulk tank milk and whey starter was collected on the same day, twice a week, for a total of 35 samples per group and analyzed by a qualified lab (Artest Spa, Modena, Italy). Milk samples were analyzed for fat, crude protein, casein, total lactose, SCC and urea content, Total Bacteria Count (**TBC**), pH, titratable acidity (°SH/50ml) and clotting time (r’) through lactodynamographic analysis (**LDG**). 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. Urea content was determined by differential pH-metry with CL-10 Plus (BioControl System, USA) according to ISO14637:2004 and SCC and TBC by flow cytometry (Schmidt-Madsen, 1975) with Combifoss and Bactoscan FC apparatus, respectively (Foss Eletric, Hillerød, Denmark) according to ISO13366-2:2006 and ISO16297:2013. Titratable acidity was determined by Soxhlet-Henkel method (Anonymous, 1963) and pH measurements using a potentiometric technique with Compact Titrator equipped with electrode P/N 53 64 (Crison Instruments, Barcelona, Spain). pH was determined at samples temperature of 25 °C after calibration of pH meter at the same temperature. Coagulation properties were assessed with a Formagraph apparatus (Foss Eletric, Hillerød, Denmark) under isothermal conditions at 35 °C (Annibaldi et al., 1977).

Whey starter samples were analyzed for titratable acidity, fermentative activity at 45, 52 and 54 °C. Acidification rate at different temperatures was evaluated by inoculating 1.5 ml of whey in 50 ml of skimmed milk (Oxoid, Termo Fisher Scientific Inc., Monza, Italy). The incubation was carried out at different temperatures (45, 52, and 54 °C) for 4 h. The acidification rate at a specific temperature was expressed as the difference between the final and initial acidity (Δ°SH.50 mL−1) (Reverberi et al., 2009).

Total amount of lactic acid bacteria (**LAB**) of whey starter was determined by dilution of the sample in physiological solution (9 g·L−1 of NaCl). Then, samples were plated in MRS agar

(Oxoid, Termo Fisher Scientific Inc., Monza, Italy) and incubated anaerobically at 45 °C for 96 h for thermophilic LAB quantification.

The amount of whole and skimmed milk coming respectively from the milking of the morning and evening in the cooking vat was recorded daily by the cheesemaker and the ratio between them was evaluated.

All cheese wheels produced during the trial were evaluated over different time points during the maturation period. Cheeses were weighed after 36 hours and 18±1 months since production in order to assess cheese yield calculated as kg of cheese/100 kg of milk in the vat. For this purpose, all the milk added and cooked in each copper vat was measured by a magnetic flowmeter (Danfoss MAGFLOW® Flowmeter Type MAG 6000) and recorded every day, together with the vat number and the code of the cheese wheels produced in that vat. At 6 months of age, X-ray analysis of all cheese produced was performed by Artest S.p.A. in order to identify internal defects like swellings, splits and “eyes”. Defects were classified as “minor”, “mild” or ”severe” based on their number and severity.

At 12 months of ripening, experts of Parmigiano Reggiano Consortium evaluated every cheese visually and by beating-hammer examination during the mandatory quality inspection as defined in the Consortium marking regulation. Following this inspection, cheese wheels were classified into different categories depending on the presence of surface or texture defects, as prescribed in the Consortium marking regulation: 1^{st} quality cheese, cheese with minor defects, 2^{nd} quality cheese and rejected cheese that cannot be marked as Parmigiano Reggiano cheese (Consorzio del Formaggio Parmigiano Reggiano, 2011)

At the end of the ripening period, 18±1 months, a representative sample of first quality cheese (24/group) were sampled according to IDF sampling procedure (Emmons, 2000) and evaluated for composition, fatty acid profile and organoleptic analysis.

Chemical analysis of cheese was performed by Artest S.p.A. for the determination of moisture (ISO 5534:2004), fat (ISO 1735:2004), and protein content (ISO 8968-1:2014), Total and water soluble nitrogen (ISO 27871:2011), volatile fatty acids and ripening index (N sol/N tot *100).

The amount of acetic, propionic and butyric acids was assessed by HPLC analysis (UV detector, SUPELcogEL C-610H 300x7.8mm column, mobile phase: 0.1% w/v phosphoric acid.).

Fatty acids methyl esters were evaluated by the Animal Production and Food Safety laboratory of the Department of Veterinary Medical Sciences, University of Bologna, by capillary gas-chromatography (Antongiovanni et al., 2007). Lipids extraction was performed by Folch method (Folch et al., 1957) while acid-catalyzed transmethylation was performed according to Stoffel method (Stoffel et al., 1959) in order to recover also the free fatty acids component of ripened cheese (Liu, 1994).

Sensory analysis of cheese was performed by CRPA (Research Center for Animal Production, Reggio Emilia, Italy) applying a Quantitative Descriptive Analysis test (QDA) in order to determine the complete sensory profile of cheese, considering view, olfaction, taste, aftertaste and structure. The test was conducted according to EN ISO 13299 (EN ISO, 2010), by 12 selected and trained panelists (ISO, 1993 and 1994).

The evaluation was performed by each panelist on two replicates of each sample served at a fixed temperature of 16±2 °C following a blind random order. Parameters evaluated are shown in Table 2. Each feature was evaluated using a graduated scale from 1 (= absence of sensation) to 7 (= highest intensity of sensation).

Statistical analysis

Summary statistics including mean, standard deviation, minimum and maximum values were calculated for all outcome parameters, stratified on treatment group. Plots of the distribution of the outcome variables, as well as Shapiro-Wilk test, were performed to determine normal distribution. Somatic cell count data were first transformed in linear Somatic Cell Score (**SCS**) (Wiggans and Shook, 1987). One-way ANOVA with treatment as fixed effects were used when the outcome variable was approximately normally distributed. Results of X-ray analysis and Consortium's evaluation were tested using Chi-square test.

For all analysis, level of significance was set for $P \leq 0.05$.

RESULTS AND DISCUSSION

Milk production

Average daily milk production (kg) was 1626.4 ± 220.1 for CTR group and 1154.9 ± 64.5 for TRT group. This difference was due to the different number of animals in the two groups present in the farm throughout the trial: 51.8 ± 7.0 cows in control group and 29.9 ± 1.5 in treated group. This situation was required by the experimental design that aimed to have in the treated group the maximum concentration of cows within 95 days since treatment administration (80%), in order to highlight any possible effects on milk and cheese quality. In this way, control milk exceeded the capacity of the cooking vat, so after the sampling procedure for the analysis, part of this milk was processed separately from the rest of the experimental milk.

Considering the number of cows in each group, average production per head was higher in TRT than CTR group (38.50 ± 1.48 vs 31.37 ± 1.47, kg), but as the production performances were not considered among the objectives of the trial, the collection of these data were not included in the experimental design, therefore comparison of individual milk yield cannot be properly analyzed.

Milk and whey starter quality

Results of milk analysis are reported in table 3**.** Overall, bulk tank milk quality did not differ between the groups except for SCS, titratable acidity and casein content percentage. Fat content (%) and coagulation time (LDG, r’) were not affected by the treatment. The effect of monensin on milk fat content is inconsistent in the published literature (Duffield et al., 2012). Some authors attribute the decrease in milk fat synthesis sometimes observed when using monensin, to a reduction in acetic acid produced in the rumen as a consequence of monensin action on ruminal microflora (Ramanzin et al., 1997; Van der Werf et al., 1998; Phipps et al., 2000). Other authors have found no effect on milk composition (Mullins et al., 2012), while Rico (Rico et al., 2014) suggested that monensin could interact with dietary component, such as starch or PUFA, when fed at high levels. Thus, the absence of monensin impact on milk fat observed in the current study, could be related to the low dietary inclusion of starch, typical of rations fed in Parmigiano Reggiano area.

Clotting time (LDG, r') of milk was not affected by the treatment, despite the differences between the two groups in casein content, titratable acidity and SCS. These results agree with the only other study that considered cheese-making properties of milk. Bertoni and collaborators (Piccioli Cappelli et al., 1996) evaluated the effects of monensin, as a feed additive on coagulation properties of milk, showing no effects on coagulation time (r'), curd firmness (a30) or on curd firming time (k20).

Despite differences shown in table 3, titratable acidity and casein content percentage of milk of both groups remained within a good range of milk used for Parmigiano Reggiano production (Zannoni and Mora, 1993; Sandri et al., 2001; Malacarne et al., 2006).

In his meta-analysis Duffield (Duffield et al., 2008a) reported heterogeneous results regarding protein content in different studies, with an overall prevalence of studies that reported a decrease in protein percentage and an increase in protein yield in cows treated with monesin.

In our study, the difference in milk protein percentage between the groups was not significant, while the reduction in casein content percentage was. Only few studies, before ours, evaluated the effects of monensin on casein content and they did not show any variation (Gandra et al., 2010; Trevisi et al., 2015). At the same time, other studies reported a significant reduction in milk protein and fat percentage that was explained by dilution effects due to the increased milk production of monensin treated cows (Phipps et al., 2000). Somatic cells were significantly lower in the treated group and this difference could be related to a better health status of animals treated with monensin (Duffield et al., 2008b).

Results of whey starter quality are shown in table 4. No important differences appeared in the activity of treated and control whey starter. The amount of lactic bacteria was not different between the groups and, indeed, the power of acidification of whey starter, here represented by fermentation activities, was not impaired. Fermentative activities are strictly related to the microbial population of whey starters and they were not affected by the treatment, as demonstrated by the high values of acidification rate (Reverberi et al., 2009). Titratable acidity of the treated group was lower than the control, but always remained within the optimal range (29-31.5 °SH/50ml) for Parmigiano Reggiano production (Reverberi et al., 2009; Gatti et al., 2014). These results are extremely important for the dairy industry as, to our knowledge, no previous studies have evaluated the effects of monensin on whey starter quality and activity.

Cheese production and defects.

During the study, 552 cheese wheels were produced, corresponding to 2 “twin” cheese wheels/group/day. As reported in table 5, the weight of twin cheese evaluated at 36 hours and 18±1 months of ripening were significantly lower (P<0.01) in TRT than CTR group (90.8 vs 93.7 kg at 36h and 79.3 vs 82.0 kg at 18 months).

Cheese yield (%), calculated as kg of cheese obtained by 100 kg of milk in the vat, showed the same difference both at 36 hours (8.6 TRT vs 8.9 CTR, %, P <0.05) and after 18 months of ripening (7.5 TRT vs 7.7 CTR, %, P <0.01).

The lower cheese yield of treated group milk could be related to its lower casein content. Cheese yield and casein content of milk are directly proportional (Fossa et al., 1994). Formaggioni et al. (2015) proposed a simple predictive formula for Parmigiano Reggiano cheese yield at 24h, including only milk fat and casein content, that has a high correlation with the actual cheese yield (Formaggioni et al., 2015).

No early swelling, detectable within 24-48 hours from production, was evident and both the experimental groups showed a very low percentage of defective cheese at 6 and 12 months of ripening (table 6).

At X-ray analysis, performed on all cheese at 6 months of ripening, 94% of cheese wheels in the treated group showed no defects, versus 88% of those in the control group. Overall, the treated group showed less ($P<0.05$) minor (6.2 TRT vs 9.4 CTR, %) mild (0 TRT vs 0.4 CTR, %) and severe (0 TRT vs 2.5 CTR, %) defects than the control group.

X-ray analysis has been demonstrated to be a useful non-destructive method to monitor the development of individual cheese during the ripening period (Kraggerud et al., 2009).

Similar results were obtained during the subsequent examination of cheese, performed at 12 months of ripening by the Consortium of Parmigiano Reggiano.

The 98.6% of cheese produced by TRT group showed no defects and was marked as 1^{st} quality cheese compared to 93.5% in the CTR group. In the TRT group, 1.4% of wheels were marked as 2^{nd} quality and none of them were rejected, while in the CTR group, 5.4% were 2^{nd} quality cheese and 1.1% were rejected (table 6). At official Consortium evaluation, defective cheeses in both groups were less than those recorded by the Consortium of Parmigiano Reggiano in the last three years (2015-2017) of production: 91.5% of 1^{st} category cheese, 7% of 2^{nd} category and 1.5% of rejected cheese (unpublished data, Consortium of Parmigiano Reggiano).

Early swelling occurs rapidly after cheese production and is due to the proliferation of gas-producing bacteria within the cheese, coliform or heterofermentative lactic acid bacteria, and more rarely, yeasts (Walstra et al., 1978).

In particular, these defects become serious in the presence of large microbial populations (10^5–10^6 /ml) and insufficient or slow acidification of milk that may occur as a consequence of a poorly active whey starter, presence of antibiotics, or contamination with phages. In order to avoid these abnormal fermentations and to assure a good ripening process, an active and proper microbial population of whey starter is fundamental (Bergère and Lenoir, 2000).

Cheese composition and sensory analysis.

After 18±1 months of ripening, cheese produced by the two groups differed for two characteristics: fat percentage was higher in treated cheese (%, 48.86 TRT vs 47.58 CTR, $P<0.05$), while soluble nitrogen and ripening index (NS/NT, %) were lower (NS g/100mg, 1.42 TRT vs 1.50 CTR, $P<0.05$; %, 29.35 TRT vs 30.69 CTR, $P<0.05$). Complete results are shown in table 5.

Cheese fat and protein content of both groups differed with the average values expected in 18 months aged Parmigiano Reggiano cheese, being fat content higher than protein content.

In a survey by Tosi et al. (2008), authors reported that the 40.5% of analyzed cheese had a fat content percentage higher than 44%, with an average of 45.28% on DM basis, and a standard deviation of 0.95. In the cited work, considering a normal distribution of this specific data subset, 95% of the samples had up to 47% of fat on DM basis, while 99% of samples reached the 48% of DM. These data are consistent with those observed in the current study and represents the actual trend of cheese-makers to produce a more fatty cheese, in order to obtain higher cheese yields. Indeed, in order to correct this trend, in

March 2018 the Consortium of Parmigiano Reggiano released a new version of the Official Regulation (Consorzio del Formaggio Parmigiano Reggiano 2018, by virtue of the Ministerial Decree in force since May 9th 2018), in which the fat : protein ratio in vat milk has been fixed to a maximum value of 1.1. In the previous version (Consorzio del Formaggio Parmigiano Reggiano, 2011), no reference values for fat and protein content of cheese were included, except for the minimum value of fat (32% of DM).

The ripening index (Nsol/Ntot,%) represents the amount of casein solubilized by proteolytic enzymes during the ripening process (Tosi et al., 2008). The entity of proteolysis is driven by several environmental and technological factors, including duration of ripening, season, and by the presence of catalytic enzymes in milk and starters used in the cheese making process (Addeo et al., 1988; Sousa et al., 2001). Among the latest, plasmin and other proteases derived from somatic cells in milk and lactic bacteria present in the whey starter, are the most effective in Parmigiano Reggiano proteolysis (Sousa et al., 2001). In the present study, environmental factors and the amount of lactic bacteria of whey starter were equal between the treatments, thus the lower amount of Nsol of TRT cheeses could be explained by the lower content of somatic cells present in milk produced by treated cows (Table 3).

As shown in table 5, acetic and propionic acids were not different between the groups. Unwanted bacteria produce propionic acid during the aging process and its presence is responsible for texture defects of cheese and undesirable flavors (Bergère and Lenoir, 2000). Also butyric acid producing clostridia are responsible for off-flavors and cheese defects. Their capability to convert lactate into butyrate, acetate, H_2 and CO_2 can lead to the accumulation of gas in the cheese matrices that results in the formation of cracks, slits and eyes (Sheehan, 2011; Brändle et al., 2016). During the ripening process, butyric acid is mainly produced by lipolysis facilitated by lipase present in cheese (Brändle et al., 2016). In our study, its amount was significantly lower ($P<0.001$) in TRT cheese than in CTR, but its value remained for both groups within the values typical of 18 months aged Parmigiano Reggiano cheeses (table 5) (Tosi et al., 2008).

These differences agree with the results of sensory analysis that showed an overall comparable profile between cheeses with a few exceptions, shown in Table 7 and Figure 2. TRT cheese samples showed a slower ripening process indicated by higher intensity of butter and sweet aroma (p.ts, 3.2 vs 3.0, $P<0.01$ and 3.5 vs 3.4, $P<0.05$), lower rind and spicy flavors (p.ts, 2.0 vs 2.1, $P<0.05$ and 1.8 vs 1.9, $P<0.05$) and higher elasticity (p.ts, 2.5 vs 2.4, $P<0.05$). In addition, TRT cheeses had a less intense, negative aroma, such as pungent, acetic and "stall", than CTR cheeses (p.ts, 2.1 vs 2.2, $P<0.05$)

However, it should be noticed that these differences did not influence the overall sensory profile of cheese of both groups which were comparable with organoleptic chracteristics of 18 months aged Parmigiano Reggiano cheese (Garavaldi et al., 2010), and in compliance with those required by the official certification body of Parmigiano Reggiano (OCQPR, 2015).

Cheese fatty acids (**FA**) profile is shown in table 8.

In the treated group, the percentage of middle-chain fatty acids (C10 to C14) on total FA was reduced (TRT 20.22 vs CTR 21.73, $P<0.05$) while among long chain fatty acids, C18:1 (TRT 22.77 vs CTR 20.79, $P<0.001$) and C:17 (TRT 0.66 vs CTR 0.61, $P<0.05$) were increased. Along with this, unsaturated (**UFA**) and saturated (**SFA**) fatty acid ratios were increased in the treated group (UFA/SFA, TRT 0.42 vs CTR 0.39, $P<0.05$).

Regardless of treatment or control, cheese fatty acid composition of all samples were in agreement with those reported by other authors for Parmigiano Reggiano cheese (Prandini et al., 2007; Mordenti et al., 2015).

Even if no other studies, to our knowledge, evaluated the effects of monensin on cheese fatty acid concentration, our results correspond with literature evaluating fatty acid variations in milk produced by cows treated with monensin sodium when administered as a feed additive or as CRC (Duffield et al., 2008a; De Marchi et al., 2015).

It has to be noticed that fatty acid composition of milk is influenced also by the stage of lactation of cows. In our study, days in milk of the experimental groups were not controlled, therefore it is possible that at least some of the difference in fatty acid profile of cheese between the groups could be due to the presence of a higher percentage of fresh cows in the treated group. Existing literature, however, supports the theory that monensin influences fatty acid concentration in milk by altering ruminal microbiota (Bell et al., 2006; McCarthy et al., 2018).

Odongo and collaborators (Odongo et al., 2007) showed an increased concentration of long chain polyunsaturated fatty acids (**PUFA**) and total monounsaturated FA (**MUFA**) in milk by 9 and 5 % respectively, in a group fed TMR + 24 mg of monensin premix per kg of DM compared to a control group. Other studies, as reported by Duffield et al. (2008b), showed the same increase in total C18:1 and PUFA concentrations, a reduction of short and medium-chain fatty acids and a reduction of PUFA/SFA ratio (AlZahal et al., 2008; De Marchi et al., 2015). The same effects were observed by in vitro studies, reporting a decrease of C18:2 ruminal biohydrogenation by lowering C18:0 production and increasing C18:1 concentration (Fellner et al., 1997; Jenkins et al., 2003).

In addition, an increase of CLA is reported after monensin supplementation (Duffield et al., 2008a), while in our study, CLA concentration remained similar between the groups (TRT 0.36 vs CTR 0.35, P >0.05). Only few recent researches, on the contrary, reported no (do Prado et al, 2015) or minimal (Akins et al., 2014) effects of monensin on milk fatty acid composition.

The rate of ruminal biohydrogenation of unsaturated fatty acids depends primarily on ruminal conditions, including microbial growth, rumen pH, and feed passage rate. Low rumen pH and altered microbial growth contribute to reduce rumen lipolysis and therefore the availability of carboxyl groups for the biohydrogenation of unsaturated fatty acids (Jenkins, 1993). Indeed, ionophores reduce rumen lipolysis, like other antimicrobial compounds known to be active mainly against gram-positive bacteria (Russell and Strobel, 1989; Van Nevel and Demeyer, 1995). However, as reported by Fellner (Fellner et al., 1997) these bacteria are not involved in rumen lipolysis neither in the last step of biohydrogenation of linoleic acid to stearic. For this reason, it seems to be possible that these molecules exert their effects also against gram negative bacteria, by changing their metabolic properties with a consequent alteration of rumen lipolysis and biohydrogenation (Newbold et al., 1993; Odongo et al., 2007).

CONCLUSIONS

Milk and whey starter produced during the trial were not affected by the treatment of cows with monensin CRC: the differences found in titratable acidity and casein content of milk and in titratable acidity of whey starter agree with the existing literature that relates these effects to the higher milk production of monensin treated cows. However, both milk and whey starter maintained the optimum quality for Parmigiano Reggiano cheese production. In particular, fermentative activities of whey starter were not impaired in the treated group at 45°C or at 54°C: this was one of the major initial concerns, considering the absence of published studies and the importance of whey starter for Parmigiano Reggiano production, in which the use of any other kind of ferments is not allowed.

After ripening, the percentage of defective cheeses in both groups was consistent with values reported by the Consortium of Parmigiano Reggiano for the last three years. Additionally, the treated group cheeses showed less defects than controls.

Chemical analysis did not highlight any negative influence of the treatment on composition and fatty acid profile. Sensory analysis demonstrated that the treatment did not substantially affect organoleptic characteristics of 18 months aged Parmigiano Reggiano cheese.

In conclusion, high quality cheese production was maintained in both control and treated group and considering our results, it is possible to state that the preventative treatment of ketosis with monensin CRC of periparturient dry cows did not impair Parmigiano Reggiano cheese quality, composition and sensory characteristics.

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Table 1. Ingredients and chemical composition (% DM) of diets fed to lactating cows of Treated[1] and Control groups

Ingredients	% (DM)
Grass hay	17.18
Wheat Straw	3.44
Alfalfa hay	27.49
Corn meal fine	3.44
Sorghum meal fine	18.90
Wheat meal fine	11.34
Wheat Bran	7.56
Protein supplement	0.94
Mineral & vitamin supplement	0.94
Chemical composition	**% (DM)**
DM, %	77.77
Crude Protein	16.11
Starch	25.05
aNDFom[2]	28.91
ADF	23.30
ADL	4.21
Fat	2.19
Ash	9.49

[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.
[2] aNDFom: alpha-amylase treated NDF, ash corrected.

Table 2. Cheese sensorial descriptors evaluated during a Quantitative Descriptive Analysis test performed by a trained expert Panel on Control and Treated[1] cheese samples at 18±1 months of ripening

Descriptor	
Visual	Color, color homogeneity, number of eyes/break, diameter, visual suitability
Aroma	Total intensity, butter smell, rind smell, vegetables smell, dried fruit smell, negative smells, flavor suitability
Taste	Sweet, salted, bitter, spicy, butter taste, rind taste, dried fruit taste, broth taste, nutmeg taste, negative flavors, suitability taste.
Texture	Elasticity, friability, humidity, solubility, granularity, suitability structure.

[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.

Table 3. Morning bulk tank milk composition and quality of Treated[1] and Control group, analyzed twice a week for a total amount of 35 samples per group

Item	Control	Treated	sem
Fat, %	3.45	3.45	0.02
Casein, %	2.51***	2.44***	0.01
Crude Protein, %	3.30	3.21	0.04
Lactose[2], %	4.78	4.79	0.03
Urea, mg/100ml	19.69	20.05	0.32
SCS, points	4.06***	3.40***	0.05
Titratable acidity, °SH/50ml	3.69***	3.61***	0.01
pH[3]	6.67	6.67	0.00
LDG[4], r'	17.67	17.27	0.23
TBC[5], *1000 UFC/ml	6.71	5.57	0.56

*** P<0.001

[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.

[2] expressed on anhydrous basis

[3] samples temperature 25°C.

[4] clotting time (min.) evaluated through lactodynamographic analysis.

[5] total bacterial count.

Table 4. Whey starter quality of Treated[1] and Control group, analyzed twice a week for a total amount of 35 samples per group

Item	Control	Treated	sem
Titratable acidity, °SH/50ml	30.43 *	29.44 *	0.23
Fermentative activity 45°C, (Δ°SH/50 mL^{-1})	2.51	2.67	0.08
Fermentative activity 52°C, (Δ°SH/50 mL^{-1})	1.93	1.97	0.05
Fermentative activity 54°C, (Δ°SH/50 mL^{-1})	1.47	1.46	0.03
Lactic Bacteria, *million UFC/ml	660.57	613.43	14.19

* $P<0.05$

[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.

Table 5. Weight, cheese yield, composition and volatile fatty acids content (acetic, butyric and propionic) of cheese produced by Control and Treated[1] milk, analyzed at 18±1 months of ripening by an accredited laboratory for Parmigiano Reggiano analysis (Artest S.p.A.)

Item	Samples, n		Average		sem
	Control	Treated	Control	Treated	
Weight 36 hrs, kg[2]	276	276	93.71^{***}	90.75^{***}	0.222
Cheese yield 36 hrs, %	276	276	8.85^{***}	8.59^{***}	0.018
Weight 18 months, kg[2]	238	254	81.98^{***}	79.34^{***}	0.193
Cheese yield 18 months, %	238	254	7.72^{***}	7.49^{***}	0.016
Skimmed:whole milk ratio	138	138	0.68	0.69	0.014
Moisture, %	24	24	30.75	30.85	0.076
Fat, % DM	24	24	47.58^{*}	48.86^{*}	0.228
Protein, %DM	24	24	45.14	44.61	0.208
NT[3], g/100g of cheese	24	24	4.9	4.83	0.023
NS [4], g/100g of cheese	24	24	1.5^{*}	1.42^{*}	0.019
NS/NT [5], %	24	24	30.69^{*}	29.35^{*}	0.361
Volatile fatty acids, mg/100g of cheese[6]	24	24			
Acetic acid			98.87	103	4.627
Butyric acid			37.3^{***}	28.56^{***}	1.499
Propionic acid			0.79	0.94	0.302

[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.
[2] Weight of two twin cheese wheels.
[3]NT= Total nitrogen
[4]NS= Water Soluble Nitrogen
[5]=Ripening index
[6] Volatile fatty acids assessed by HPLC analysis
* P<0.05
*** P<0.001

Table 6. Evaluation of cheese produced by Treated[1] and Control group, performed after 6 months of ripening by X-ray and after 12 months by visual and beating hammer (Official expertisation of Consortium).

	Control	Treated	χ^2
Cheese, n	276	276	
X-ray analysis (6 months), %			
No defects	87.7*	93.8*	0.59
Minor defects	9.4*	6.2*	0.33
Mild defects	0.4*	0.0*	0.48
Severe defects	2.5*	0.0*	0.06
Consortium evaluation (12 months), %			
First quality	93.5*	98.6*	0.67
Medium quality	5.4*	1.4*	0.07
Rejected	1.1*	0*	0.22

* $P<0.05$

[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.

Table 7. Cheese sensorial descriptors significantly different between Treated[1] and Control group, evaluated by Quantitative Descriptive Analysis test performed by a trained expert Panel on 18±1 months cheese samples (samples n 24 +24). Complete sensory profile is shown in Figure 2.

	Control	Treated	sem
Butter	3.0^{**}	3.2^{**}	0.06
Rind	2.1^{*}	2.0^{*}	0.06
Sweet	3.4^{*}	3.5^{*}	0.05
Spicy	1.9^{*}	1.8^{*}	0.06
Others[2]	2.2^{*}	2.1^{*}	0.06
Elasticity	2.4^{*}	2.5^{*}	0.07

[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.
[2] negative aroma, such as pungent, acetic and “stall”
* P<0.05
** P<0.01

Table 8. Fatty acid composition (% of fatty acids) of 18±1 months aged cheese produced with Control and Treated[1] milk (samples n, 24+24).

Fatty acid	Control	Treated	sem
C4:0	3.35	3.6	0.291
C6:0	1.51	1.44	0.118
C8:0	1.28	1.19	0.056
C10:0	3.59*	3.31*	0.095
C10:1	0.3**	0.25**	0.009
C12:0	4.23**	3.79**	0.087
C12:1	0.12**	0.1**	0.004
C14:0	12.34*	11.77*	0.164
C14:1	1.15***	1***	0.018
C15:0	1.52	1.45	0.032
C16:0	34.44	34.07	0.288
C16:1	1.47	1.4	0.08
C17:0	0.61*	0.66*	0.015
C18:0	6.84	6.97	0.155
C18:1	20.79***	22.77***	0.316
C18:2	2.14	2.16	0.056
C18:3 n3	0.54	0.5	0.02
C20:0	0.08	0.07	0.007
C20:4 n6	0.14	0.12	0.007
CLA tot	0.35	0.36	0.014
Others[2]	3.21	3.02	0.254

*P<0.05, **P <0.01, *** P<0.001

[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.

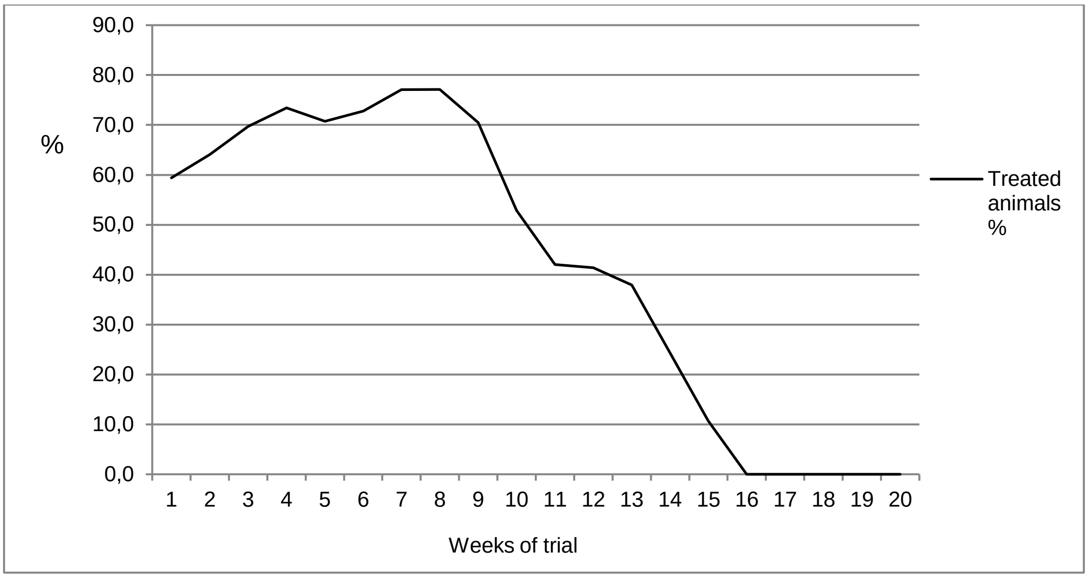
[2] Non-identified fatty acids

Figure captions

Figure 1. Percentage of animals in Treated group within 95 days since treatment[1] administration, from the 1st to the 20th week of trial.

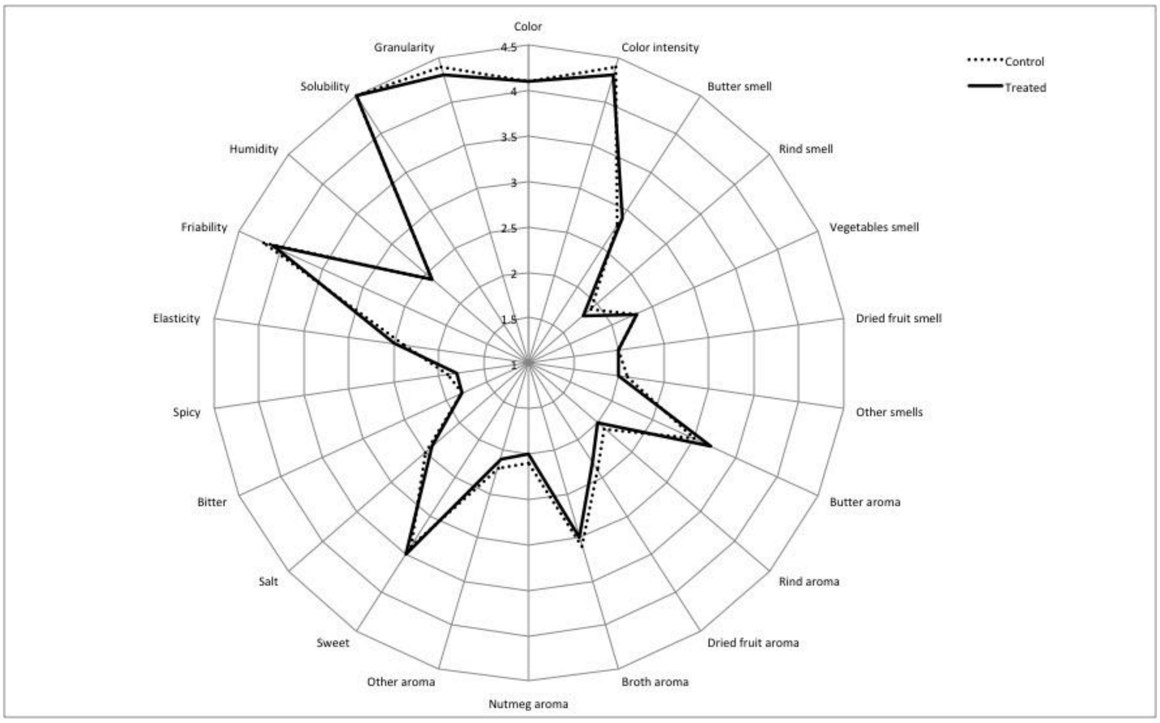
Figure 2. Sensory profile of 18±1 months aged cheese produced by Treated[1] and Control group evaluated by Quantitative Descriptive Analysis test performed by a trained expert Panel (samples, n 24 + 24).

Mammi Figure 1.



[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.

Mammi Figure 2.



[1] Treatment: monensin control release capsule, administered to cows 21 days before predicted calving date.