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Does the dry cow treatment with monensin controlled release capsule affect Parmigiano Reggiano cheese production?

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

18 Mammi

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

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

26

27 **EFFECT OF MONENSIN ON PARMIGIANO REGGIANO**

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

31

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ABSTRACT

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

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

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

56 For 20 weeks, milk obtained from each group and whey starter were separately managed
57 and transported in the cheese factory, where 2 cheese wheels per group were produced
58 daily, making 552 PR cheese wheels in total. Morning bulk tank milk composition,
59 cheesemaking properties and whey starter fermentation activities were analyzed twice a
60 week. Every aspect of the cheesemaking process was recorded and the resulting cheese was
61 evaluated after 36 hours, 6, 12 and 18 months from production for yield, texture defects,
62 composition and fatty acids profile. Milk from the two groups differed for somatic cell
63 content (TRT 3.04 vs CTR 4.06, Somatic Cell Score p.ts), total bacterial count (TRT 4.08 vs
64 CTR 6.08, *1000 UFC/ml), titratable acidity (TRT 3.66 vs CTR 3.72, °SH/50ml) and casein
65 content percentage (TRT 2.4 vs CTR 2.5, %). Whey starter parameters were comparable

66 between the two groups. Final cheese composition and organoleptic profile were not
67 influenced by the treatment except for C18:1 content being enhanced (TRT 22.8 vs CTR
68 20.8, % of fatty acids). Percentage of defected ripened cheese was significantly lower in the
69 treated group, both at x-ray evaluation performed at 6 months (TRT 6.2 vs CTR 12.3, %)
70 and at the Consortium inspection, performed at 12 months of ripening (TRT 1.5 vs CTR
71 6.5, %). On the other hand, average cheese yield at 18 months of ripening was partially
72 reduced (TRT 7.5 vs CTR 7.7, %).

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

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77 **Key words**

78 Monensin, milk quality, Parmigiano Reggiano, cheese quality

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INTRODUCTION

81

82 Ketosis is one of the most important diseases in modern herds due to its high incidence and
83 its deep impact on cow health and performance. Recent studies reported that subclinical
84 ketosis (SCK) incidence, within the first 16 days of lactation, varies from 22 to 43% in
85 European and American herds respectively (McArt et al., 2012; Suthar et al., 2013). Cows
86 affected by subclinical or clinical ketosis have a higher risk of developing pathologies such as
87 displaced abomasum and metritis as well as risk of culling as a consequence of health
88 problems (Duffield et al., 2009; McArt et al., 2012; Suthar et al., 2013). Reproductive
89 performance of these animals is often impaired and milk production reduced (McArt et al.,
90 2015) together with changed composition. Indeed, ketosis reduces the protein content of milk

91 on first DHIA test day (Vanholder et al., 2015) and may consequently impair its cheese
92 making properties.

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

96 Monensin is a carboxylic polyether ionophore commonly used as a feed additive in ruminants
97 to alter rumen fermentation in order to improve energy efficiency (Russell and Strobel,
98 1989). Its effects on energy metabolism are well known and widely described both in beef
99 and dairy cattle (Goodrich et al., 1984; Ipharraguerre and Clark, 2003; Duffield et al., 2012).

100 Monensin has a selective action on rumen microbes: it alters ion exchange through the inner
101 and outer membranes of microbial cells. In this way it reduces the prevalence of protozoa and
102 gram positive population and promotes gram negative proliferation, that is mainly
103 responsible for propionate production (Russell and Strobel, 1989). As a consequence, the
104 ratio between acetate and propionate changes in favor of propionate, thereby improving
105 energy metabolism of cows (Russell and Strobel, 1989).

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

109 In recent years, numerous studies have investigated the effects of monensin administration on
110 animal metabolism and performance and regardless of whether or not it is administered as a
111 feed additive or controlled release capsule, the beneficial effects have included reduced
112 NEFA and BHBA plasma concentration, increased propionate production in the rumen and
113 decreased incidence of clinical and subclinical ketosis (Duffield et al., 1998). On the other
114 hand, only a few studies have explored the effects on milk quality and these have shown
115 contrasting results. No studies, to our knowledge, have assessed the impact of monensin on

116 cheese quality. Mullins (Mullins et al., 2012) did not find any changes in milk production and
117 composition in monensin treated cows, while other authors found a significant reduction in
118 milk fat and protein content percentage (Odongo et al., 2007; Duffield et al., 2012).

119 Parmigiano Reggiano cheese is traditionally made with raw, unpasteurized and partially
120 skimmed milk. To produce this kind of cheese, feedstuff, management and milk processing
121 must be in compliance with Parmigiano Reggiano regulations (Consorzio del Formaggio
122 Parmigiano Reggiano, 2011) by virtue of the Ministerial Decree in force since October 1st
123 2011, that implement the European regulation for PDO production (Council Regulation, n
124 510/2006). Cows are fed without silages and therefore, in order to maintain milk production
125 and composition and to avoid ruminal disorders, a proper inclusion of high quality hays in the
126 ration is always needed (Fustini et al., 2017).

127 In this specific manufacturing process, milk composition and environmental wild microflora
128 are extremely important (Mordenti et al., 2017). Indeed, microbial population of whey starter
129 is fundamental for the quality and the maturation process of the cheese (Coloretti et al.,
130 2016). Considering its antimicrobial activity, some have suggested that the administration of
131 monensin might potentially impair cheese composition and quality. Therefore, the main
132 purpose of our study was to evaluate the effect of a mass treatment of dry cows with
133 monensin CRC on Parmigiano Reggiano cheese production.

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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

160 29-30 cows per group and to have at least 1000-1100 kg of milk/day/group, sufficient to
161 produce 2 cheese wheels a day from each group.

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

169

170 ***Milking and cheese production***

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

176 Each day, 2 cheese wheels per group were produced and marked following Parmigiano
177 Reggiano cheese production standards (Consorzio del Formaggio Parmigiano Reggiano,
178 2011). Cheese wheels of both groups were stored together in the same traditional ripening
179 rooms for 18 months.

180

181 ***Milk, whey starter and cheese analysis***

182 Every day the amount of milk produced and delivered to the cheese factory by the two groups
183 was recorded. Morning bulk tank milk and whey starter was collected on the same day, twice
184 a week, for a total of 35 samples per group and analyzed by a qualified lab (Artest Spa,

185 Modena, Italy). Milk samples were analyzed for fat, crude protein, casein, total lactose, SCC
186 and urea content, Total Bacteria Count (**TBC**), pH, titratable acidity ($^{\circ}\text{SH}/50\text{ml}$) and clotting
187 time (r') through lactodynamographic analysis (**LDG**). Milk components were measured by
188 mid-infrared analysis (Biggs, 1978) with MilkoScan 6000 FT (Foss Eletric, Hillerød,
189 Denmark). Precalibration procedures were performed according to International Dairy
190 Federation Standards 141C:2000 (IDF, 2000), using total nitrogen for protein expression.
191 Urea content was determined by differential pH-metry with CL-10 Plus (BioControl System,
192 USA) according to ISO14637:2004 and SCC and TBC by flow cytometry (Schmidt-Madsen,
193 1975) with Combifoss and Bactoscan FC apparatus, respectively (Foss Eletric, Hillerød,
194 Denmark) according to ISO13366-2:2006 and ISO16297:2013. Titratable acidity was
195 determined by Soxhlet-Henkel method (Anonymous, 1963) and pH measurements using a
196 potentiometric technique with Compact Titrator equipped with electrode P/N 53 64 (Crison
197 Instruments, Barcelona, Spain). pH was determined at samples temperature of 25 °C after
198 calibration of pH meter at the same temperature. Coagulation properties were assessed with a
199 Formagraph apparatus (Foss Eletric, Hillerød, Denmark) under isothermal conditions at 35
200 °C (Annibaldi et al., 1977).

201 Whey starter samples were analyzed for titratable acidity, fermentative activity at 45, 52 and
202 54 °C. Acidification rate at different temperatures was evaluated by inoculating 1.5 ml of
203 whey in 50 ml of skimmed milk (Oxoid, Termo Fisher Scientific Inc., Monza, Italy). The
204 incubation was carried out at different temperatures (45, 52, and 54 °C) for 4 h. The
205 acidification rate at a specific temperature was expressed as the difference between the final
206 and initial acidity ($\Delta^{\circ}\text{SH}.50 \text{ mL}^{-1}$) (Reverberi et al., 2009).

207 Total amount of lactic acid bacteria (**LAB**) of whey starter was determined by dilution of the
208 sample in physiological solution ($9 \text{ g}\cdot\text{L}^{-1}$ of NaCl). Then, samples were plated in MRS agar

209 (Oxoid, Termo Fisher Scientific Inc., Monza, Italy) and incubated anaerobically at 45 °C for
210 96 h for thermophilic LAB quantification.

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

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

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

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

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

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

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

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

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

255

256 *Statistical analysis*

257 Summary statistics including mean, standard deviation, minimum and maximum values

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

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

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RESULTS AND DISCUSSION

268

269 *Milk production*

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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.

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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.

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Milk and whey starter quality

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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

291 when using monensin, to a reduction in acetic acid produced in the rumen as a consequence
292 of monensin action on ruminal microflora (Ramanzin et al., 1997; Van der Werf et al., 1998;
293 Phipps et al., 2000). Other authors have found no effect on milk composition (Mullins et al.,
294 2012), while Rico (Rico et al., 2014) suggested that monensin could interact with dietary
295 component, such as starch or PUFA, when fed at high levels. Thus, the absence of monensin
296 impact on milk fat observed in the current study, could be related to the low dietary inclusion
297 of starch, typical of rations fed in Parmigiano Reggiano area.

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

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

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

311 In our study, the difference in milk protein percentage between the groups was not
312 significant, while the reduction in casein content percentage was. Only few studies, before
313 ours, evaluated the effects of monensin on casein content and they did not show any
314 variation (Gandra et al., 2010; Trevisi et al., 2015). At the same time, other studies reported

315 a significant reduction in milk protein and fat percentage that was explained by dilution
316 effects due to the increased milk production of monensin treated cows (Phipps et al., 2000).
317 Somatic cells were significantly lower in the treated group and this difference could be
318 related to a better health status of animals treated with monensin (Duffield et al., 2008b).

319 Results of whey starter quality are shown in table 4. No important differences
320 appeared in the activity of treated and control whey starter. The amount of lactic bacteria
321 was not different between the groups and, indeed, the power of acidification of whey starter,
322 here represented by fermentation activities, was not impaired. Fermentative activities are
323 strictly related to the microbial population of whey starters and they were not affected by
324 the treatment, as demonstrated by the high values of acidification rate (Reverberi et al.,
325 2009). Titratable acidity of the treated group was lower than the control, but always
326 remained within the optimal range (29-31.5 °SH/50ml) for Parmigiano Reggiano production
327 (Reverberi et al., 2009; Gatti et al., 2014). These results are extremely important for the
328 dairy industry as, to our knowledge, no previous studies have evaluated the effects of
329 monensin on whey starter quality and activity.

330

331 ***Cheese production and defects.***

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

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

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

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

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

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

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

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

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

366 In particular, these defects become serious in the presence of large microbial populations
367 (10^5 – 10^6 /ml) and insufficient or slow acidification of milk that may occur as a consequence
368 of a poorly active whey starter, presence of antibiotics, or contamination with phages. In
369 order to avoid these abnormal fermentations and to assure a good ripening process, an
370 active and proper microbial population of whey starter is fundamental (Bergère and Lenoir,
371 2000).

372

373 ***Cheese composition and sensory analysis.***

374 After 18 ± 1 months of ripening, cheese produced by the two groups differed for two
375 characteristics: fat percentage was higher in treated cheese (% , 48.86 TRT vs 47.58 CTR,
376 $P < 0.05$), while soluble nitrogen and ripening index (NS/NT, %) were lower (NS g/100mg,
377 1.42 TRT vs 1.50 CTR, $P < 0.05$; %, 29.35 TRT vs 30.69 CTR, $P < 0.05$). Complete results
378 are shown in table 5.

379 Cheese fat and protein content of both groups differed with the average values expected in
380 18 months aged Parmigiano Reggiano cheese, being fat content higher than protein content.

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

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

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

405 As shown in table 5, acetic and propionic acids were not different between the groups.
406 Unwanted bacteria produce propionic acid during the aging process and its presence is
407 responsible for texture defects of cheese and undesirable flavors (Bergère and Lenoir,
408 2000). Also butyric acid producing clostridia are responsible for off-flavors and cheese
409 defects. Their capability to convert lactate into butyrate, acetate, H_2 and CO_2 can lead to the
410 accumulation of gas in the cheese matrices that results in the formation of cracks, slits and
411 eyes (Sheehan, 2011; Brändle et al., 2016). During the ripening process, butyric acid is
412 mainly produced by lipolysis facilitated by lipase present in cheese (Brändle et al., 2016). In

413 our study, its amount was significantly lower ($P<0.001$) in TRT cheese than in CTR, but its
414 value remained for both groups within the values typical of 18 months aged Parmigiano
415 Reggiano cheeses (table 5) (Tosi et al., 2008).

416 These differences agree with the results of sensory analysis that showed an overall
417 comparable profile between cheeses with a few exceptions, shown in Table 7 and Figure 2.
418 TRT cheese samples showed a slower ripening process indicated by higher intensity of
419 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
420 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,
421 2.5 vs 2.4, $P<0.05$). In addition, TRT cheeses had a less intense, negative aroma, such as
422 pungent, acetic and “stall”, than CTR cheeses (p.ts, 2.1 vs 2.2, $P<0.05$)

423 However, it should be noticed that these differences did not influence the overall sensory
424 profile of cheese of both groups which were comparable with organoleptic characteristics of
425 18 months aged Parmigiano Reggiano cheese (Garavaldi et al., 2010),
426 and in compliance with those required by the official certification body of Parmigiano
427 Reggiano (OCQPR, 2015).

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

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

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

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

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

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

457 In addition, an increase of CLA is reported after monensin supplementation (Duffield et al.,
458 2008a), while in our study, CLA concentration remained similar between the groups (TRT
459 0.36 vs CTR 0.35, $P > 0.05$). Only few recent researches, on the contrary, reported no (do
460 Prado et al, 2015) or minimal (Akins et al., 2014) effects of monensin on milk fatty acid
461 composition.

462 The rate of ruminal biohydrogenation of unsaturated fatty acids depends primarily on
463 ruminal conditions, including microbial growth, rumen pH, and feed passage rate. Low
464 rumen pH and altered microbial growth contribute to reduce rumen lipolysis and therefore
465 the availability of carboxyl groups for the biohydrogenation of unsaturated fatty acids
466 (Jenkins, 1993). Indeed, ionophores reduce rumen lipolysis, like other antimicrobial
467 compounds known to be active mainly against gram-positive bacteria (Russell and Strobel,
468 1989; Van Nevel and Demeyer, 1995). However, as reported by Fellner (Fellner et al.,
469 1997) these bacteria are not involved in rumen lipolysis neither in the last step of
470 biohydrogenation of linoleic acid to stearic. For this reason, it seems to be possible that
471 these molecules exert their effects also against gram negative bacteria, by changing their
472 metabolic properties with a consequent alteration of rumen lipolysis and biohydrogenation
473 (Newbold et al., 1993; Odongo et al., 2007).

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CONCLUSIONS

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

485 After ripening, the percentage of defective cheeses in both groups was consistent with
486 values reported by the Consortium of Parmigiano Reggiano for the last three years.
487 Additionally, the treated group cheeses showed less defects than controls.
488 Chemical analysis did not highlight any negative influence of the treatment on composition
489 and fatty acid profile. Sensory analysis demonstrated that the treatment did not substantially
490 affect organoleptic characteristics of 18 months aged Parmigiano Reggiano cheese.
491 In conclusion, high quality cheese production was maintained in both control and treated
492 group and considering our results, it is possible to state that the preventative treatment of
493 ketosis with monensin CRC of periparturient dry cows did not impair Parmigiano Reggiano
494 cheese quality, composition and sensory characteristics.

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498

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503

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REFERENCIES

Addeo, F., L. Moio, and C. Stingo. 1988. Typical Characteristics of Proteolysis in Parmigiano-Reggiano cheese. In: Triennial Research on the Composition and the Peculiar Characteristics of Parmigiano-Reggiano Cheese. Consorzio Parmigiano Reggiano, Reggio Emilia, Italy.

Akins, M.S., K.L. Perfield, H.B. Green, S.J. Bertics, and R.D. Shaver. 2014. Effect of monensin in lactating dairy cow diets at 2 starch concentrations. *J. Dairy Sci.* 97:917–929. <https://doi:10.3168/jds.2013-6756>.

AlZahal, O., N.E. Odongo, T. Mutsvangwa, M.M. Or-Rashid, T.F. Duffield, R. Bagg, P. Dick, G. Vessie, and B.W. McBride. 2008. Effects of Monensin and Dietary Soybean Oil on Milk Fat Percentage and Milk Fatty Acid Profile in Lactating Dairy Cows. *J. Dairy Sci.* 91:1166–1174. <http://dx.doi.org/10.3168/jds.2007-0232>.

Anonymous. 1963. Säuregradbestimmung nach Soxhlet-Henkel (SH). Titratable acidity evaluation with the Soxhlet-Henkel (SH) method. *Milchwissenschaft* 18:520.

Annibaldi, S., G. Ferri, and R. Mora. 1977. Nuovo orientamenti nella valutazione tecnica del latte: tipizzazione lattodinamografica [New trends for milk technical evaluation: lactodynamographic standardisation]. *Sci. E Tec. Latt. Casearia* 28:115–126.

Antongiovanni, M., S. Banni, A. Buccioni, L. Cercaci, G. Contarini, M. Cutrignelli, G. Lercker, D. Lo Fiego, M. Mele, S. Minieri, A. Nudda, E. Piasentier, and A. Serra. 2007. *Metodi Di Analisi per Lo Studio Della Frazione Lipidica Del Latte, Dei Prodotti Di Origine Animale e Degli Alimenti Zootecnici*. PLUS PISA UNIVERSITY PRESS, Pisa, Italy.

Bell, J. A., J. M. Griinari, and J. J. Kennelly. 2006. Effect of safflower oil, flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. *J. Dairy Sci.* 89:733–748.

528 Bergère, J.L., and J. Lenoir. 2000. Cheese manufacturing accidents and cheese defects.
529 Lavoisier Publishing, France.

530 Biggs, D.A. 1978. Instrumental infrared estimation of fat, protein, and lactose in milk:
531 collaborative study. *J. Assoc. Off. Anal. Chem.* 61:1015–1034.

532 Brändle, J., K.J. Domig, and W. Kneifel. 2016. Relevance and analysis of butyric acid
533 producing clostridia in milk and cheese. *Food Control* 67:96–113.
534 <https://doi:10.1016/j.foodcont.2016.02.038>.

535 Coloretti, F., C. Chiavari, M. Nocetti, P. Reverberi, E. Bortolazzo, V. Musi, and L. Grazia.
536 2016. Whey starter addition during maturation of evening milk: effects on some
537 characteristics of cheese milk and Parmigiano–Reggiano cheese. *Dairy Sci. &*
538 *Technol.* 96:185–197.

539 Consorzio del Formaggio Parmigiano Reggiano. 2011. Specification of the Parmigiano
540 Reggiano cheese. Accessed Oct. 24 2017.
541 http://www.parmigianoreggiano.com/consortium/rules_regulation_2/default.aspx

542 Consorzio del Formaggio Parmigiano Reggiano. 2018. Specification of the Parmigiano
543 Reggiano cheese. Accessed May 2018.
544 [https://www.parmigianoreggiano.it/consorzio/disciplinare_produzione_vigente_30_0](https://www.parmigianoreggiano.it/consorzio/disciplinare_produzione_vigente_30_03_2018/default.aspx)
545 [3_2018/default.aspx](https://www.parmigianoreggiano.it/consorzio/disciplinare_produzione_vigente_30_03_2018/default.aspx)

546 Council Regulation (EC) No 510/2006 of 20 March 2006 on the protection of geographical
547 indications and designations of origin for agricultural products and foodstuffs.
548 Official Journal of the European Union. 49:12-25. Accessed Oct. 2017. [http://eur-](http://eur-lex.europa.eu/eli/reg/2006/510/oj)
549 [lex.europa.eu/eli/reg/2006/510/oj](http://eur-lex.europa.eu/eli/reg/2006/510/oj)

550 De Marchi, F.E., J.V. Romero, J.C. Damasceno, P.A. Grande, L.M. Zeoula, and S. Dos.
551 2015. Pelleting in associated with sodium monensin increases the conjugated linoleic

552 acids concentration in the milk of dairy cows fed canola seeds. *Asian-Australas. J.*
553 *Anim. Sci.* 28:1095–1104. <https://doi:10.5713/ajas.14.0865>.

554 Do Prado, R.M., C. Côrtes, C. Benchaar, and H.V. Petit. 2015. Interaction of sunflower oil
555 with monensin on milk composition, milk fatty acid profile, digestion, and ruminal
556 fermentation in dairy cows. *Anim. Feed Sci. Technol.* 207:85–92.
557 doi:10.1016/j.anifeedsci.2015.06.017.

558 Duffield, T.F., D. Sandals, K.E. Leslie, K. Lissemore, B.W. McBride, J.H. Lumsden, P. Dick,
559 and R. Bagg. 1998. PHYSIOLOGY AND MANAGEMENT:-Efficacy of Monensin
560 for the Prevention of Subclinical Ketosis in Lactating Dairy Cows. *J. Dairy Sci.*
561 81:2866–2873.

562 Duffield, T.F., A.R. Rabiee, and I.J. Lean. 2008a. A Meta-Analysis of the Impact of
563 Monensin in Lactating Dairy Cattle. Part 2. Production Effects. *J. Dairy Sci.* 91:1347–
564 1360. <https://doi:10.3168/jds.2007-0608>.

565 Duffield, T.F., A.R. Rabiee, and I.J. Lean. 2008b. A Meta-Analysis of the Impact of
566 Monensin in Lactating Dairy Cattle. Part 3. Health and Reproduction. *J. Dairy Sci.*
567 91:2328–2341. <https://doi:10.3168/jds.2007-0801>.

568 Duffield, T.F., K.D. Lissemore, B.W. McBride, and K.E. Leslie. 2009. Impact of
569 hyperketonemia in early lactation dairy cows on health and production. *J. Dairy Sci.*
570 92:571–580. <https://doi:10.3168/jds.2008-1507>.

571 Duffield, T.F., A. Rabiee, and I.J. Lean. 2012. Overview of Meta-Analysis of Monensin in
572 Dairy Cattle. *Vet. Clin. North Am. Food Anim. Pract.* 28:107–119.
573 <https://doi:10.1016/j.cvfa.2011.12.009>.EN ISO, 2010. Sensory analysis.
574 Methodology. General guidance for establishing a sensory profile. EN ISO 13299.
575 European Committee for Standardization. Bruxelles, Belgium

576 EMA, 2013. European Public Assessment Reports (EPAR) European Medicine Agency.
577 Accessed January 2018
578 [http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/veterinary/medicines/
579 002235/vet_med_000267.jsp&mid=WC0b01ac058001fa1c](http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/veterinary/medicines/002235/vet_med_000267.jsp&mid=WC0b01ac058001fa1c)

580 Emmons, D.B., 2000. Sampling and analysis. Pages 74–86 in: Practical Guide for Control of
581 Cheese Yield.. International Dairy Federation, Brussels, Belgium.

582 Fellner, V., F.D. Sauer, and J.K.. Kramer. 1997. Effect of Nigericin, Monensin, and
583 Tetronasin on Biohydrogenation in Continuous Flow-Through Ruminal
584 Fermenters.pdf. *J. Dairy Sci.* 80:921–928.

585 Folch, J., M. Lees, and G.H. Sloane Stanley. 1957. A simple method for the isolation and
586 purification of total lipids from animal tissues. *J Biol Chem* 226:497–509.

587 Formaggioni, P., A. Summer, M. Malacarne, P. Franceschi, and G. Mucchetti. 2015. Italian
588 and Italian-style hard cooked cheeses: Predictive formulas for Parmigiano-Reggiano
589 24-h cheese yield. *Int. Dairy J.* 51:52–58. <https://doi:10.1016/j.idairyj.2015.07.008>.

590 Fossa, E., M. Pecorari, S. Sandri, F. Tosi, and P. Mariani. 1994. The role of milk casein
591 content in the Parmigiano-Reggiano cheese production: chemical composition, rennet
592 coagulation properties and dairy tech- nological behaviour of milk. *Sci. E Tec. Latt.*
593 *Casearia* 45:519–535.

594 Fustini, M., A. Palmonari, G. Canestrari, E. Bonfante, L.M.E. Mammi, M.T. Pacchioli,
595 G.C.J. Sniffen, R.J. Grant, K.W. Cotanch, and A. Formigoni. 2017. Effect of
596 undigested neutral detergent fiber content of alfalfa hay on lactating dairy cows:
597 Feeding behavior, fiber digestibility, and lactation performance. *J. Dairy Sci.*
598 100:4475–4483. <https://doi:10.3168/jds.2016-12266>.

599 Gandra, J.R., F.P. Rennó, J.E. de Freitas Júnior, M.V. dos Santos, and A.P.C. de Araújo.
600 2010. Productive performance and milk protein fraction composition of dairy cows
601 supplemented with sodium monensin. *Rev. Bras. Zootec.* 39:1810–1817.

602 Garavaldi, A., M. Zannoni, B. Giussani, S. Roncoroni, L. Galassi, and M. Turrini. 2010.
603 Scheda sensoriale per il Parmigiano Reggiano: scelta dei descrittori e messa a punto
604 del profilo. *Sci. E Tec. Latt. Casearia* 61:367–369.

605 Gatti, M., B. Bottari, C. Lazzi, E. Neviani, and G. Mucchetti. 2014. Invited review: Microbial
606 evolution in raw-milk, long-ripened cheeses produced using undefined natural whey
607 starters. *J. Dairy Sci.* 97:573–591. <https://doi:10.3168/jds.2013-7187>.

608 Goodrich, R.D., J.E. Garrett, D.R. Gast, M.A. Kirick, D.A. Larson, and J.C. Meiske. 1984.
609 Influence of monensin on the performance of cattle. *J. Anim. Sci.* 58:1484–1498.

610 IDF, 1993. Milk: Determination of Nitrogen Content, IDF Standard 20B:1993, Parts 1 and 2.
611 Int. Dairy Fed., Brussels, Belgium.

612 IDF, 2000. Whole milk: Determination of milk fat, protein and lactose content – guidance on
613 the operation of mid-infrared instruments. IDF Standard 141C:2000, Int. Dairy Fed.,
614 Brussels, Belgium.

615 ISO, 1993. Sensory analysis. General guidance for the selection, training and monitoring of
616 assessors. Part 1: Selected assessors. ISO 8586-1. International Organisation for
617 Standardization. Geneva, Switzerland. ISO, 1994. Sensory analysis. General
618 guidance for the selection, training and monitoring of assessors. Part 2: Experts.
619 ISO 8586-2. International Organisation for Standardization. Geneva,
620 Switzerland. ISO, 2004. Cheese and processed cheese products. Determination of
621 fat content. Gravimetric method (Reference method). ISO 1735. International
622 Organisation for Standardization. Geneva, Switzerland.

623 ISO, 2004. Cheese and processed cheese. Determination of the total solids content (Reference
624 method). ISO 5534. International Organisation for Standardization. Geneva,
625 Switzerland.

626 ISO, 2004. Milk. Determination of urea content. Enzymatic method using difference in pH
627 (Reference method). ISO 14637. International Organisation for Standardization.
628 Geneva, Switzerland.

629 ISO, 2006. Milk. Enumeration of somatic cells. Part 2: Guidance on the operation of fluoro-
630 opto-electronic counters. ISO 13366-2. International Organisation for
631 Standardization. Geneva, Switzerland.

632 ISO, 2011. Cheese and processed cheese. Determination of the nitrogenous fractions. ISO
633 27871. International Organisation for Standardization, Geneva, Switzerland.

634 ISO, 2013. Milk. Bacterial count: Protocol for the evaluation of alternative methods. ISO
635 16297. International Organisation for Standardization. Geneva, Switzerland.

636 ISO, 2014. Milk and milk products. Determination of nitrogen content. Part 1: Kjeldahl
637 principle and crude protein calculation. ISO 8968-1. International Organisation for
638 Standardization. Geneva, Switzerland.

639 Ipharraguerre, I.R., and J.H. Clark. 2003. Usefulness of ionophores for lactating dairy cows: a
640 review. *Anim. Feed Sci. Technol.* 106:39–57. [https://doi:10.1016/S0377-](https://doi:10.1016/S0377-8401(03)00065-8)
641 [8401\(03\)00065-8](https://doi:10.1016/S0377-8401(03)00065-8).

642 Jenkins, T.C. 1993. Lipid metabolism in the rumen. *J. Dairy Sci.* 76:3851–3863.

643 Jenkins, T.C., V. Fellner, and R.K. McGuffey. 2003. NUTRITION, FEEDING, AND
644 CALVES-Monensin by Fat Interactions on Trans Fatty Acids in Cultures of Mixed
645 Ruminal Microorganisms Grown in Continuous Fermentors Fed Corn or Barley. *J.*
646 *Dairy Sci.* 86:324–330.

647 Kraggerud, H., J.P. Wold, M. Høy, and R.K. Abrahamsen. 2009. X-ray images for the
648 control of eye formation in cheese. *Int. J. Dairy Technol.* 62:147–153.
649 <https://doi:10.1111/j.1471-0307.2009.00478.x>.

650 Liu, K.-S. 1994. Preparation of fatty acid methyl esters for gas-chromatographic analysis of
651 lipids in biological materials. *J. Am. Oil Chem. Soc.* 71:1179–1187.

652 Malacarne, M., A. Summer, E. Fossa, P. Formaggioni, P. Franceschi, M. Pecorari, and P.
653 Mariani. 2006. Composition, coagulation properties and Parmigiano-Reggiano cheese
654 yield of Italian Brown and Italian Friesian herd milks. *J. Dairy Res.* 73:171.
655 <https://doi:10.1017/S0022029905001688>.

656 McArt, J.A.A., D.V. Nydam, and G.R. Oetzel. 2012. Epidemiology of subclinical ketosis in
657 early lactation dairy cattle. *J. Dairy Sci.* 95:5056–5066. [https://doi:10.3168/jds.2012-](https://doi:10.3168/jds.2012-5443)
658 5443.

659 McArt, J.A.A., D.V. Nydam, and M.W. Overton. 2015. Hyperketonemia in early lactation
660 dairy cattle: A deterministic estimate of component and total cost per case. *J. Dairy*
661 *Sci.* 98:2043–2054. <https://doi:10.3168/jds.2014-8740>. McCarthy M.M., T. R.
662 Overton, G. D. Mechor, D. E. Bauman, T. C. Jenkins and D. V. Nydam. 2018. Short
663 communication: Field study to investigate the associations between herd-level risk
664 factors for milk fat depression and bulk tank milk fat percent in dairy herds feeding
665 monensin. *J. Dairy Sci.* 101:3118–3125. <https://doi.org/10.3168/jds.2017-13932>

666 Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in
667 feeds with refluxing in beakers or crucibles: collaborative study. *J. AOAC Int.*
668 85:1217–1240.

669 Mordenti, A.L., N. Brogna, F. Merendi, G. Canestrari, M. Dall’Olio, G. Biagi, and A.
670 Formigoni. 2015. Effect of feeding whole soybean and linseed on milk and
671 Parmigiano-Reggiano cheese lipid fraction.. *Prog. Nutr.* 17:220–230.

672 Mordenti, A.L., N. Brogna, and A. Formigoni. 2017. Review: The link between feeding dairy
673 cows and Parmigiano-Reggiano cheese production area. *Prof. Anim. Sci.* 33:520–529.
674 <https://doi:10.15232/pas.2016-01602>.

675 Mullins, C.R., L.K. Mamedova, M.J. Brouk, C.E. Moore, H.B. Green, K.L. Perfield, J.F.
676 Smith, J.P. Harner, and B.J. Bradford. 2012. Effects of monensin on metabolic
677 parameters, feeding behavior, and productivity of transition dairy cows. *J. Dairy Sci.*
678 95:1323–1336. <https://doi:10.3168/jds.2011-4744>.

679 Newbold, C.J., R.J. Wallace, and N.D. Walker. 1993. The effect of tetronasin and monensin
680 on fermentation, microbial numbers and the development of ionophore-resistant
681 bacteria in the rumen. *J. Appl. Bacteriol.* 75:129–134.

682 OCQPR. 2015. Scheda per la valutazione sensoriale del Parmigiano-Reggiano in pezzi.
683 Allegato 1 a Piano di controllo DOP Parmigiano Reggiano. Organismo Controllo
684 Qualità Produzioni Regolamentate, Reggio Emilia, Italy. Accessed May 2018.
685 [http://www.ocqpr.it/images/documentazione/204_Scheda_sens._formaggio_in_pezzi_](http://www.ocqpr.it/images/documentazione/204_Scheda_sens._formaggio_in_pezzi_-Allegato_1_PC_-_Rev._10.07.15_def.pdf)
686 [- Allegato 1 PC - Rev. 10.07.15 def.pdf](http://www.ocqpr.it/images/documentazione/204_Scheda_sens._formaggio_in_pezzi_-Allegato_1_PC_-_Rev._10.07.15_def.pdf)

687 Odongo, N.E., M.M. Or-Rashid, R. Bagg, G. Vessie, P. Dick, E. Kebreab, J. France, and
688 B.W. McBride. 2007. Long-Term Effects of Feeding Monensin on Milk Fatty Acid
689 Composition in Lactating Dairy Cows. *J. Dairy Sci.* 90:5126–5133.
690 <https://doi:10.3168/jds.2007-0242>.

691 Phipps, R.H., J.I.D. Wilkinson, L.J. Jonker, M. Tarrant, A.K. Jones, and A. Hodge. 2000.
692 Effect of monensin on milk production of Holstein-Friesian dairy cows. *J. Dairy Sci.*
693 83:2789–2794.

694 Piccioli Cappelli, F., M.G. Maianti, and G. Bertoni. 1996. Effect of Monensin
695 supplementation on milk and metabolic characteristics of dairy cows. Pages 561–
696 562 S.I.S. Vet, Perugia.

697 Prandini, A., S. Sigolo, G. Tansini, N. Brogna, and G. Piva. 2007. Different level of
698 conjugated linoleic acid (CLA) in dairy products from Italy. *J. Food Compos. Anal.*
699 20:472–479. <https://doi:10.1016/j.jfca.2007.03.001>.

700 Ramanzin, M., L. Bailoni, S. Schiavon, and G. Bittante. 1997. Effect of Monensin on Milk
701 Production and Efficiency of Dairy Cows Fed Two Diets Differing in Forage to
702 Concentrate Ratios¹. *J. Dairy Sci.* 80:1136–1142.

703 Reverberi, P., G. Gambini, A. Caroli, A. Pecorari, and M. Nocetti. 2009. Profilo dei siero-
704 innesti per Parmigiano- Reggiano e modalità analitiche di valutazione [Profile and
705 analytical valuation of Parmigiano-Reggiano whey starters]. *Sci. E Tec. Latt. Casearia*
706 60:37–42.

707 Rico, D.E., A.W. Holloway, and K.J. Harvatine. 2014. Effect of monensin on recovery from
708 diet-induced milk fat depression. *J. Dairy Sci.* 97:2376–2386.

709 Russell, J.B., and H.J. Strobel. 1989. Effect of ionophores on ruminal fermentation. *Appl.*
710 *Environ. Microbiol.* 55:1.

711 Sandri, S., F. Tosi, M.S. Mariani, P. Vecchia, M. Malacarne, and A. Summer. 2001.
712 Osservazioni sull'andamento delle principali caratteristiche casearie del latte per
713 Parmigiano-Reggiano durante gli anni 1990. *Ann Fac Med Vet Univ. Parma* 21:235–
714 247.

715 Schmidt-Madsen, P. 1975. Fluoro-opto-electronic cell-counting on milk.. *J. Dairy Res.*
716 42:227–239.

717 Sheehan, J.J. 2011. *Cheese. Avoidance of gas blowing.* Academic Press, San Diego, CA.

718 Sousa, M.J., Y. Ardö, and P.L.H. McSweeney. 2001. Advances in the study of proteolysis
719 during cheese ripening. *Int. Dairy J.* 11:327–345. doi:10.1016/S0958-6946(01)00062-
720 0.

721 Stoffel, W., F. Chu, and J.E.H. Ahrens. 1959. Analysis of Long-Chain Fatty Acids by Gas-

- 722 Liquid Chromatography. *Anal. Chem.* 31:307–308.
- 723 Suthar, V.S., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of
724 subclinical ketosis and relationships with postpartum diseases in European dairy
725 cows. *J. Dairy Sci.* 96:2925–2938. <https://doi:10.3168/jds.2012-6035>.
- 726 Tosi, F., S. Sandri, G. Tedeschi, M. Malacarne, and E. Fossa. 2008. Variazioni di
727 composizione e proprietà fisico-chimiche del Parmigiano-Reggiano durante la
728 maturazione e in differenti zone della forma. *Sci. E Tec. Latt. Casearia* 59:507–528.
- 729 Trevisi, E., F. Piccioli Cappelli, M. Mezzetti, G. Lovotti, and P. Bani. 2015. Effect of the
730 ruminal slow-release of monensin during the transition period of dairy cows on health
731 status, energy metabolism and inflammatory conditions. *Ital. J. Anim. Sci.* 14:9.
- 732 UNI, 1998. Dairy products. General directives for the nitrogen determination according to
733 Kjeldahl method. UNI 10760. Ente di Normazione Italiano. Milano, Italy.
- 734 Van der Werf, J.H.J., L.J. Jonker, and J.K. Oldenbroek. 1998. Effect of monensin on milk
735 production by Holstein and Jersey cows. *J. Dairy Sci.* 81:427–433.
- 736 Vanholder, T., J. Papen, R. Bemers, G. Vertenten, and A.C.B. Berge. 2015. Risk factors for
737 subclinical and clinical ketosis and association with production parameters in dairy
738 cows in the Netherlands. *J. Dairy Sci.* 98:880–888. doi:10.3168/jds.2014-8362.
- 739 Van Nevel, C., and D.I. Demeyer. 1995. Lipolysis and Biohydrogenation of Soybean Oil in
740 the Rumen In Vitro: Inhibition by Antimicrobials. *J. Dairy Sci.* 78:2797–2806.
741 [https://doi:10.3168/jds.S0022-0302\(95\)76910-7](https://doi:10.3168/jds.S0022-0302(95)76910-7).
- 742 Walstra, P., A. Noomen, and T.J. Geurts. 1978. Major cheese groups. Elsevier Applied
743 Science, London, UK.
- 744 Wiggans, G.R., and G.E. Shook. 1987. A lactation measure of somatic cell count. *J. Dairy*
745 *Sci.* 70:2666–2672.
- 746 Zannoni, M., and R. Mora. 1993. Evolution of the milk quality program for the Parmigiano-

747 Reggiano cheese. *Il Latte*, 18:572–581.

748

749 **Table 1.** Ingredients and chemical composition (% DM) of diets fed to lactating cows of Treated¹
 750 and Control groups

751	Ingredients	% (DM)
752	Grass hay	17.18
	Wheat Straw	3.44
753	Alfalfa hay	27.49
	Corn meal fine	3.44
754	Sorghum meal fine	18.90
	Wheat meal fine	11.34
755	Wheat Bran	7.56
	Protein supplement	0.94
756	Mineral & vitamin supplement	0.94
757	Chemical composition	% (DM)
	DM, %	77.77
	Crude Protein	16.11
758	Starch	25.05
	aNDFom ²	28.91
759	ADF	23.30
	ADL	4.21
	Fat	2.19
760	Ash	9.49

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

763 ² aNDFom: alpha-amylase treated NDF, ash corrected.

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765 **Table 2.** Cheese sensorial descriptors evaluated during a Quantitative Descriptive Analysis test
 766 performed by a trained expert Panel on Control and Treated¹ cheese samples at 18±1 months of
 767 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.

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

770

771 **Table 3.** Morning bulk tank milk composition and quality of Treated¹ and Control group, analyzed
 772 twice a week for a total amount of 35 samples per group
 773

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
Titrateable 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

774 ^{***} $P < 0.001$

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

777 ² expressed on anhydrous basis

778 ³ samples temperature 25°C.

779 ⁴ clotting time (min.) evaluated through lactodynamographic analysis.

780 ⁵ total bacterial count.

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Table 4. Whey starter quality of Treated¹ and Control group, analyzed twice a week for a total amount of 35 samples per group

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

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* $P < 0.05$

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

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Table 5. Weight, cheese yield, composition and volatile fatty acids content (acetic, butyric and propionic) of cheese produced by Control and Treated¹ 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

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

² Weight of two twin cheese wheels.

³NT= Total nitrogen

⁴NS= Water Soluble Nitrogen

⁵=Ripening index

⁶ Volatile fatty acids assessed by HPLC analysis

* $P < 0.05$

*** $P < 0.001$

803 **Table 6.** Evaluation of cheese produced by Treated¹ and Control group, performed after 6 months of
804 ripening by X-ray and after 12 months by visual and beating hammer (Official expertisation of
805 Consortium).

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	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

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820 * $P < 0.05$

821 ¹ Treatment: monensin control release capsule, administered to cows
822 21 days before predicted calving date.
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824 **Table 7.** Cheese sensorial descriptors significantly different between Treated¹ and Control group,
 825 evaluated by Quantitative Descriptive Analysis test performed by a trained expert Panel on 18±1
 826 months cheese samples (samples n 24 +24). Complete sensory profile is shown in Figure 2.
 827

	Control	Treated	sem
829 Butter	3.0**	3.2**	0.06
830 Rind	2.1*	2.0*	0.06
831 Sweet	3.4*	3.5*	0.05
832 Spicy	1.9*	1.8*	0.06
833 Others ²	2.2*	2.1*	0.06
834 Elasticity	2.4*	2.5*	0.07

836
 837 ¹ Treatment: monensin control release capsule, administered to cows 21 days before predicted
 838 calving date.

839 ² negative aroma, such as pungent, acetic and “stall”

840 * $P < 0.05$

841 ** $P < 0.01$

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843 **Table 8.** Fatty acid composition (% of fatty acids) of 18±1 months aged cheese produced with
 844 Control and Treated¹ milk (samples n, 24+24).
 845

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

846 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

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

849 ² Non-identified fatty acids
 850

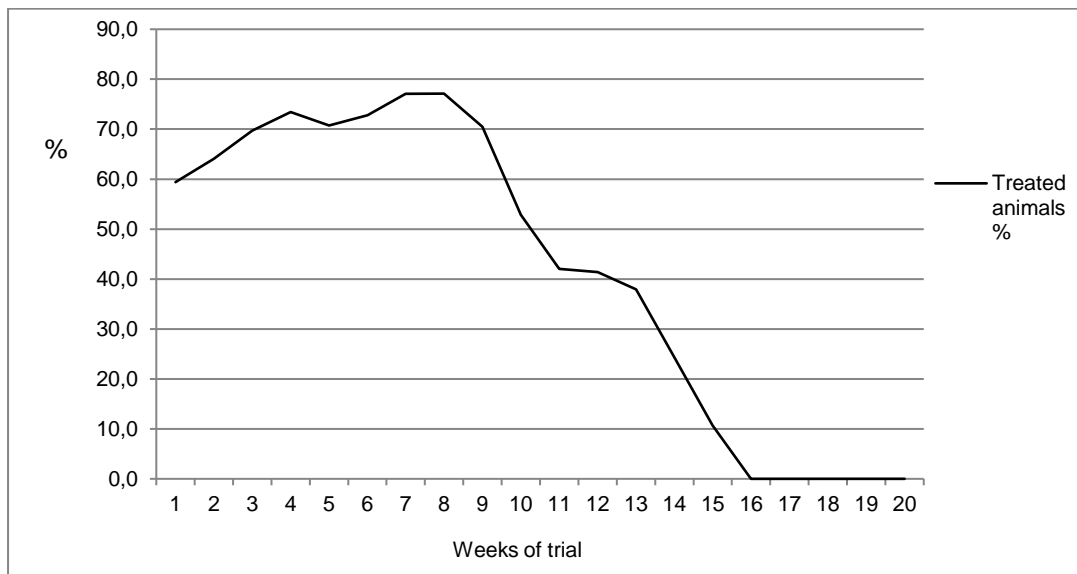
851 **Figure captions**

852 **Figure 1.** Percentage of animals in Treated group within 95 days since treatment¹
853 administration, from the 1st to the 20th week of trial.

854 **Figure 2.** Sensory profile of 18±1 months aged cheese produced by Treated¹ and Control
855 group evaluated by Quantitative Descriptive Analysis test performed by a trained expert
856 Panel (samples, n 24 + 24).

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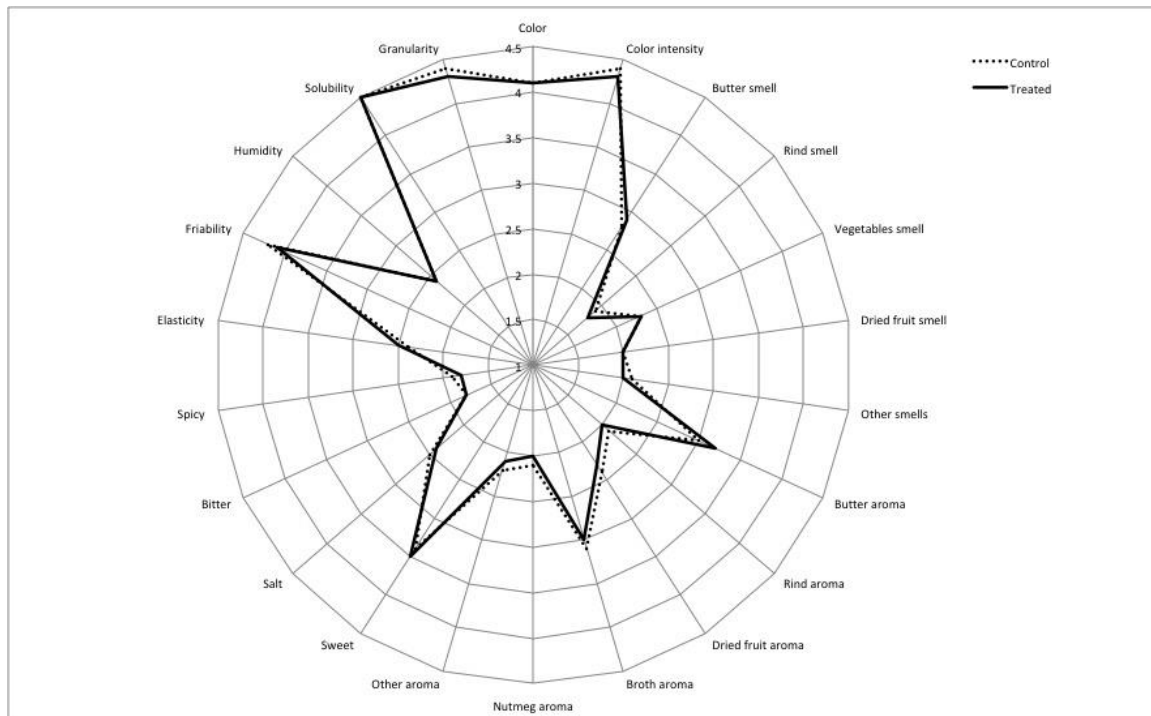
858 **Mammi Figure 1.**



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860 ¹ Treatment: monensin control release capsule, administered to cows 21 days before predicted calving
861 date.
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863 Mammi Figure 2.



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