

# Effect of controlled-release monensin on automatically registered body condition score, milk $\beta$ -hydroxybutyrate, milk yield and milk lactate dehydrogenase in fresh dairy cows

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## Abstract

The aim of this study was to compare the effect of controlled-release monensin on the automatic registered body condition score (BCS), and biomarkers registered using a fully automated inline analyzer, such as milk  $\beta$ -hydroxybutyrate (BHB), milk yield (MY) and milk lactate dehydrogenase (LDH).

Two experimental groups were formed: (1) monensin group (GK) supplemented with monensin (a monensin controlled release capsule (MCRC) of 32.4 g, n = 42) and (2) control group (GO) (capsule containing no monensin, n = 42). Treatment began 21 days before calving, and the experiment was finished one month after calving. In order to gather data about MY, BHB, and LDH, Herd Navigator a real-time analyzer (Lattec I/S, Hillerød, Denmark) was used together with a DeLaval milking robot (DeLaval Inc., Tumba, Sweden). BCS was measured using 3D BCS cameras (DeLaval, DeLaval International AB). All data were registered at one, 15 and 30 days after calving. The statistical analysis was performed using SPSS 26.0 (SPSS Inc., Chicago, USA) package. It was concluded that in the group of cows with monensin supplement (a monensin controlled release capsule of 32.4 g), the body condition score was statistically significantly higher at the 15th (+0.24, p=0.003) and 30th (+0.52, p<0.001) days after calving, the productivity of cows in this group increased by 10.25% from the 1st to the 15th day and by 22.49% from the beginning of the experiment to the 30th day (p<0.001), lactate dehydrogenase activities at the 15th and 30th days after calving in this group were lower (p<0.001), and also in this group, the number of cows with a value of  $\beta$ -hydroxybutyrate of 0.06 mmol/L decreased from the beginning of the experiment to 30 days after calving by 4.70% (from 19.00% to 14.30%) compared with the control group.

**Key words:** ionophore, ketosis, mastitis, milk yield, sensors, transition

## Introduction

The potentialities of technology are now affecting the agriculture and cattle sectors. Technology support and Precision Livestock Farming concepts positively influence for livestock farming progress, as they can trigger efficiency gains, waste reduction, and environmental sustainability improvements. The main goal of these technological instruments is not to substitute, but rather to help, a farmer who still remains the most important aspect of good animal management. Multiple sensors, a data infrastructure, and data analytics are examples of novel digital equipment that can be used to monitor animals and their environments (Buller et al. 2020). Technological advances have been made that offer aid in in-line body condition score (BCS), and milk  $\beta$ -hydroxybutyrate (BHB), milk yield (MY) and milk lactate dehydrogenase (LDH) estimation (Rodriguez et al. 2020). A completely automated inline LDH, progesterone, and BHB analyzer that may be paired with a milking robot is available on the commercial market for even better herd assessment. Using its precision diagnostic methods, we can gain a better grasp of the current parameters influencing dairy cow health (Hommeida et al. 2004). DeLaval Corporate designed the first commercially available 3D BCS system based on image processing technologies (Somers et al. 2015).

The risk of many peri-partum diseases of dairy cows is influenced considerably by the nutritional and metabolic status of the animal and in particular poor adaptation to negative energy balance is associated with an increased danger of subsequent diseases (Herdt. 2000). According to LeBlanc (2010) most dairy cattle in the first weeks after calving are suffering from reduced feed intake, negative energy balance, hypocalcaemia, decreased immune function, and bacterial contamination of the uterus. Registration of body condition score (BCS) and its change during the transition to lactation serves as an indicator of body fat stores and mobilization (Rathbun et al. 2017)  $\beta$ -hydroxybutyrate (BHB) was linearly decreased as BCS loss decreased and has been reported as a risk of ketosis. BCS loss was demonstrated to increase BHB post calving compared with animals that maintained BCS (Sheehy et al. 2017). The body condition score of a dairy cow is an assessment of the proportion of body fat that it possesses, and it is recognized by animal scientists and producers as being an important factor in dairy cattle management (Roche et al. 2009). Cows with a higher BCS than 4.0 at calving had higher circulating concentrations of NEFA in early lactation compared with cows with moderate or low BCS (Pires et al. 2013). Immune cells are exposed to high NEFA and BHB concentrations during the after-calving period, which has been shown

to be associated with decreased neutrophil function (Suriyasathaporn et al. 2000). Suriyasathaporn et al. (2000) proposed that hyperketonemia leads to the udder defect by affecting leukocyte phagocytosis, cytokine production, and migration. Hammon et al. (2013) found that the killing ability of neutrophils was negatively correlated with NEFA concentrations in the first week of calving. The results of Chagunda et al. (2006) suggested that the model has the potential to provide the basis for a useful decision-supporting tool for mastitis management. The analysis of real data using L-Lactate dehydrogenase (LDH) is used as the indicator in milk of clinical mastitis.

Monensin, for improvement of the health of dairy cows, is widely used in most parts of the world (Duffield et al. 2008). Monensin is a polyether ionophore antibiotic, a group of nutritional supplements in dairy cows that alters volatile fatty acid production; it stimulates propionate production and decreases acetate and butyrate production, and also increases the change in composition of free fatty acids by altering the rumen microbial flora and increasing the population of gram-negative bacteria (Benchaar et al. 2008). According to studies by Lowe (1998), these changes in the bacterial population increase energy metabolism efficiency, improve nitrogen metabolism, and reduce the risk of bloating and rumen acidosis. Stephenson et al. (1997) found that a monensin continuous-release capsule reduced serum BHB concentrations and increased of serum glucose concentrations.

According to the literature we hypothesized that controlled-release monensin has an effect on various parameters, registered by innovative technologies (with the help of multiple sensor systems) which enable automatic registration of body condition score, milk  $\beta$ -hydroxybutyrate, milk yield and milk lactate dehydrogenase in fresh dairy cows. The aim of this study was to compare the effect of controlled-release monensin on automatically registered body condition score and biomarkers registered using a fully automated inline analyzer, such as milk  $\beta$ -hydroxybutyrate, milk yield and milk lactate dehydrogenase.

## Materials and Methods

### Location and animals

This study was carried out during 2021.03.01 – 2021.09.30 at one Lithuanian dairy farm with 550 dairy cows (location - 55.911381565736, 21.881321760608195). The monensin controlled-release capsules (Kexxtone Elanco GmbH) used for the study emitted monensin at daily doses of 335 mg, when administered once with an oral balling gun in accordance with the manufac-

Table 1. Chemical composition of feeding rations for dry and fresh dairy cows.

Parameters	Units	Fresh dairy cows	Dry cows
Dry matter	%	44.0	45.0
Dry matter intake (DM)	kg DM/d	26.5	11.1
Net energy lactation	MJ/kg DM	7.01	4.20
Crude protein	g/kg DM	175	102
Crude Fat	g/kg DM	45	24
Fatty acids	g/kg DM	34	10
Protein balance in rumen	g/kg DM	22	9
Neutral detergent fiber	g/kg DM	287	632
Starch	g/kg DM	204	23

turer's instructions. Two experimental groups were formed as follows: (1) Monensin group (GK) supplemented with monensin (a monensin controlled-release capsule (MCRC) of 32.4 g, n=42) and (2) Control group (GO) (capsule containing no monensin, n=42). Treatment began 21 days before calving, and the study was finished one month after calving. All cows were multiparous. The cows were fed with a total mixed ration (TMR) at 06:00 am and 06:00 pm (twice per day) and housed in a loose house system. The feed ration was balanced to fit the energy and nutrient requirements calculated based on the NRC [16] for Holstein cows producing, on average, 40 kg/day of milk and on average 550 kg of body weight (Table 1).

The cows were milked twice per day (at 05:00 am and 05:00 pm) using a parlor system. The average energy corrected milk yield (4.15% fat, 3.6% protein) in 2020 was 10,500 kg per cow and year. During the study, contact with the animals was minimal, and animal welfare issues were avoided.

### Measurements

In order to gather data about MY, BHB, and LDH, a Herd Navigator real-time analyzer (Lattec I/S, Hillerød, Denmark) was used together with a DeLaval milking robot (DeLaval Inc., Tumba, Sweden). An inline sampler automatically took at every milking a representative sample of several millilitres of milk from each cow during the robot-milking process. The sample was then transferred into the Herd Navigator™ for further analysis. BCS was measured using 3D BCS cameras (DeLaval International AB, Sweden).

### Measurement of BHB and LDH

For measurements of BHB and LDH was used a Herd Navigator™ real-time analyzer (Lattec I/S, Hillerød, Denmark) and was combined with a DeLaval milking robot (DeLaval Inc., Tumba, Sweden) to detect milk BHB concentrations and LDH activities. During

the milking process, a sample of several millilitres of milk was taken from each cow with the help of an inline sampler in order to determine the BHB and LDH parameters. The raw measurements were corrected according to company – specified methods to take into account differences between sets of dry sticks and variations in the surrounding humidity. The most extreme outliers were then removed from the calculations. Measurements over 200  $\mu\text{mol}/\text{min}$  per litre were set to 200 (a maximum value), and all indicators above the upper limit were excluded from the study. This is how data are normalized in the Herd Navigator system. The milk yield from each cow was measured using a validated an optical milk meter. The LDH activity ( $\mu\text{mol}/\text{min}$ ) was calculated as the LDH activity divided by the milk yield from the latest milking session.

### BCS recording system

The technology behind body condition scoring is based on a 3D-camera which records certain parts of the animal: from above, the rear part of the back from the short ribs to the tail end. Every time a cow walks under the camera, the system identifies the specific movement and captures images of the cow; it then chooses the best image of the cow in the video-recording. The 3D-camera uses light coding technology, which projects a pattern of infrared ray dots on the back of the cow. Following this, the distances between these certain dots are measured; according to the manufacturer, a 3D-image of the back is modeled, and an algorithm converts that image information into a body condition score. Since the cameras were placed above separation gates near the milking robot parlor, the cow's measurements were taken every time they went to be milked. As a golden standard, the scale used to develop the algorithm was based on visual scoring using a 1-5 point scale system. In this scale, the spinous to transverse processes are assessed and given a specific score, where one corresponds to the lowest and five to the highest condition score.

Table 2. Body condition score by groups of cows.

Days after calving	Group	M	SEM	95% CI		p between groups
				Lower Bound	Upper Bound	
1	GO	3.09 <sup>a</sup>	0.06	2.98	3.21	n.s.
	GK	3.23 <sup>A</sup>	0.06	3.11	3.35	
15	GO	2.94 <sup>b</sup>	0.06	2.83	3.05	0.003
	GK	3.18 <sup>B</sup>	0.06	3.07	3.29	
30	GO	2.68 <sup>c</sup>	0.05	2.57	2.78	< 0.001
	GK	3.20 <sup>AB</sup>	0.05	3.09	3.30	

GK – monensin group; GO - control group. M – mean; SE – standard error; CI – 95% confidence interval for mean. <sup>a, b, c</sup> different letters indicates that the differences between days after calving (1, 15, 30) for the GO group are statistically significant at  $p < 0.05$ . <sup>A, B, C</sup> different letters indicate that the differences between days after calving (1, 15, 30) for the GK group are statistically significant at  $p < 0.05$ .

Table 3. Milk yield (kg/d) by groups of cows.

Days after calving	Group	M	SEM	95% CI		p between groups
				Lower Bound	Upper Bound	
1	GO	32.48 <sup>a</sup>	1.27	29.95	35.01	n.s.
	GK	33.66 <sup>A</sup>	1.26	31.16	36.16	
15	GO	29.23 <sup>b</sup>	1.07	27.10	31.37	< 0.001
	GK	37.11 <sup>B</sup>	1.06	34.99	39.22	
30	GO	32.16 <sup>a</sup>	1.04	30.09	34.23	< 0.001
	GK	41.23 <sup>C</sup>	1.03	39.19	43.28	

GK – monensin group; GO - control group. M – mean; SE - standard error; CI – 95% confidence interval for mean. <sup>a, b, c</sup> - different letters indicates that the differences between days after calving (1, 15, 30) for the GO group are statistically significant at  $p < 0.05$ . <sup>A, B, C</sup> different letters indicates that the differences between days after calving (1, 15, 30) for the GK group are statistically significant at  $p < 0.05$ .

All measurements started on the first day after calving, again at 15 days after calving and finished at 30 days after calving.

### Data analysis and statistics

Statistical analysis of the automatically registered body condition score and inline biomarker data was performed using the SPSS 26.0 (SPSS Inc., Chicago, USA) package. The normal distribution of all variables was assessed using the Shapiro-Wilk test. Milk LDH values were converted to logarithmic expressions (log<sub>10</sub>). We used the general linear repeated measures model (repeated measures with the “between subject factor” test) to compare the indicators of days (1, 15 and 30) and between GO and GK groups of cows. The results of the study were given as means and standard error (SEM) and 95% confidence interval for mean. The Bonferroni test was used to compare data by day and by group. A linear and second-order polynomial regression was used to analyse the change in the continuous variables of the automatically registered body condition score and inline biomarkers during the experiment. Milk BHB measurements were classified according to the values recorded in the study (0.04, 0.05 and 0.06 mmol/L). Fisher’s exact test was used to assess the relationship between  $\beta$ -hydroxybutyrate levels

in milk and the day after calving. The correlation of BCS with MY and LDH was calculated according to Pearson, and the correlation with BHB was calculated according to Spearman’s test. A probability of less than 0.05 was considered significant ( $p < 0.05$ ) for all tests used in this study.

### Results

The difference between the mean values of the body condition score was statistically significantly higher in the GK cows compared to the GO group at the 15th (+0.24,  $p = 0.003$ ) and 30th (+0.52,  $p < 0.001$ ) days after calving (Table 2).

The body condition score in the GO group decreased throughout the study period ( $p < 0.001$ ), while in the GK group it decreased on the 15th day after calving (-1.58%,  $p = 0.005$ ) and then increased (+0.56%,  $p = 0.040$ ) on the 30th day after calving ( $p < 0.001$ ).

The average milk yield of cows and their body condition in the GO group was significantly lower on the 15<sup>th</sup> and 30<sup>th</sup> days after calving (-7.87 kg and -9.08 kg, respectively,  $p < 0.001$ ) compared with the GK group (Table 3).

The milk yield (kg) of the GK group constantly increased ( $p < 0.001$ ). The productivity of cows in this

Table 4. Milk lactate dehydrogenase (LDH) activity ( $\mu\text{mol}/\text{min}$ ) by groups of cows.

Days after calving	Group	M	SEM	95% CI		p between groups
				Lower Bound	Upper Bound	
1	GO	1.41 <sup>a</sup>	0.03	1.36	1.47	n.s.
	GK	1.42 <sup>A</sup>	0.03	1.37	1.48	
15	GO	1.53 <sup>b</sup>	0.02	1.48	1.57	< 0.001
	GK	1.37 <sup>B</sup>	0.02	1.32	1.41	
30	GO	1.42 <sup>a</sup>	0.03	1.37	1.48	< 0.001
	GK	1.45 <sup>A</sup>	0.02	1.42	1.51	

GK – monensin group; GO – control group. M – mean; SE – standard error; CI – 95% confidence interval for mean. <sup>a, b, c</sup> different letters indicates that the differences between days after calving (1, 15, 30) for the GO group are statistically significant at  $p > 0.05$ . <sup>A, B, C</sup> different letters indicates that the differences between days after calving (1, 15, 30) for the GK group are statistically significant at  $p < 0.05$ .

Table 5. Milk  $\beta$ -hydroxybutyrate (mmol/L) by groups of cows.

Days after calving	Group	M	SEM	95% CI		p between groups
				Lower Bound	Upper Bound	
1	GO	0.060 <sup>a</sup>	0.001	0.059	0.061	< 0.001
	GK	0.048 <sup>A</sup>	0.001	0.047	0.049	
15	GO	0.057 <sup>b</sup>	0.001	0.055	0.058	< 0.001
	GK	0.049 <sup>A</sup>	0.001	0.047	0.050	
30	GO	0.054 <sup>b</sup>	0.001	0.052	0.055	< 0.001
	GK	0.049 <sup>A</sup>	0.001	0.047	0.050	

GK – monensin group; GO – control group. M – mean; SE – standard error; CI – 95% confidence interval for mean. <sup>a, b, c</sup> different letters indicates that the differences between days after calving (1, 15, 30) for the GO group are statistically significant at  $p < 0.05$ . <sup>A, B, C</sup> different letters indicates that the differences between days after calving (1, 15, 30) for the GK group are statistically significant at  $p < 0.05$ .

group increased by 10.25% from the 1st to the 15<sup>th</sup> day and by 22.49% from the beginning of the experiment to the 30<sup>th</sup> day ( $p < 0.001$ ), while in the GO group there was a decrease (-10.00 %,  $p < 0.001$ ) and a subsequent increase (+10.00 %,  $p < 0.001$ ) in milk yield ( $p < 0.001$ ).

Lactate dehydrogenase activities (Table 4) in the milk of both groups were almost the same on the first day after calving, but on the following days of the study they were lower in the GK group than in the GO group ( $p < 0.001$ ).

From the beginning to the end of the study, the LDH in the milk of GO cows increased by 19.15% ( $p > 0.001$ ), while in the GK group such an increase in this indicator was stopped.

The mean BHB value of the GO group exceeded the mean value of the GK group in all study periods (from 0.005 to 0.012 mmol/L,  $p > 0.001$ ), but the difference between the groups decreased as the number of days after calving increased (Table 5). The average value of BHB in the GK group did not change during the study, and in the GO group it decreased (from 0.060 to 0.054 mmol/L  $p > 0.001$ ).

The analysis showed that, in the GK group, the number of cows with a BHB value of 0.06 mmol/l decreased from the beginning of the experiment to 15-30 days by 4.70% (from 19.00% to 14.30%).

The body condition score of both groups of cows was weakly negatively correlated with milk yield and lactate dehydrogenase activity. The analysis showed that only lactate dehydrogenase was positively associated with the assessment of body condition score in the GK group, but all calculated Pearson correlation coefficients were statistically unreliable (Fig. 1).

After assessing the relationship between the milk  $\beta$ -hydroxybutyrate and body condition score by groups and experimental days using the Spearman correlation analysis, the coefficients obtained turned out to be close to zero and no relationship between these indicators was shown; on the other hand, it should be borne in mind that the  $\beta$ -hydroxybutyrate variation was very small.

## Discussion

This study has shown that BCS at the 15<sup>th</sup> and 30<sup>th</sup> days after calving was higher in the cows which were supplemented with monensin (a monensin controlled release capsule of 32.4 g) 21 st days before calving compared to those supplemented with capsule containing no monensin (see Table 2). Huang et al. (2019) reported that BCS is a useful method of monitoring relations among nutritional management, reproduction,

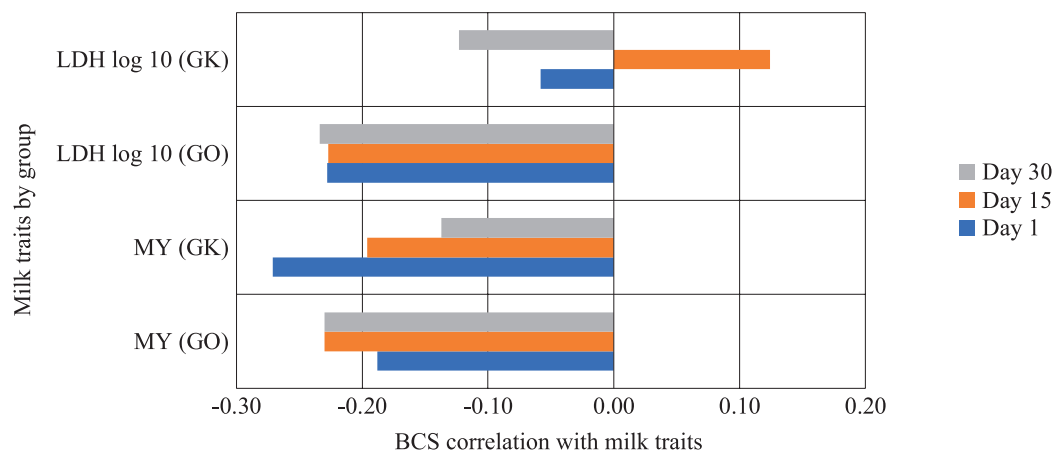


Fig. 1. Body condition score (BCS) correlation with milk traits.

GO – control group (capsule containing no monensin); GK – monensin group; BCS – body condition score; MY – milk yield (kg); LDH – milk lactate dehydrogenase activity ( $\mu\text{mol}/\text{min}$ ).

and ketosis and supporting in farm management decisions. This decrease may be a consequence of a negative energy balance (NEB) due to a rapid increase in milk production and when additional feed intake is limited. This can lead to the mobilization of body reserves and weight loss (Garro et al. 2014). The increased mobilization of NEFA and BHB production has been associated with changes in bodyweight (BW) and consequently BCS, especially for high yield milking animals (Weber et al. 2013). The evaluation of the change in BW over time as well as BCS can be used as a proxy for negative energy balance in transition cows (Caixeta et al. 2015). We determined that the BCS in the GO group decreased throughout the study period, while in the GK group it decreased on the 15th day after calving and then increased on the 30th day after calving. Our results coincide with Duffield et al. (2008), who found that monensin increased BCS by 0.03 % and similarly improved body weight change (0.06 kg/d) and the effects depend on the stage of lactation and the dose. The magnitude of the response for BCS was 0.014 to 0.053 of a BCS across all study treatment intervals. One BCS is equivalent to approximately 80 kg (Schwager-Suter et al. 2001); thus, the BCS change was approximately equal to 1.2 to 4.2 kg of BW. The main action of monensin is to increase ruminal propionate production, thereby increasing the supply of this glucogenic precursor and can equally increase ruminal propionate production (Mirzaei-Alamouti et al. 2016) and can possibly provide additional benefits for body condition (Hausmann et al. 2018).

The results showed that the average milk yield of cows, as well as the assessment of their body condition in the GO group ( $p < 0.05$ , see Table 3) was significantly lower on the 15th and 30th days after calving compared with the GK group. The productivity of cows in the GK group steadily increased. Monensin is known

to be effective in increasing the milk yield (McCarthy et al. 2015). Phipps et al. (2000) evaluated the effect of monensin on milk yield and health in lactating cows and mentioned that monensin slightly reduced dry matter intake (DMI) and increased milk yield, which ranged from 0.8 to 2.8 kg/d. Data from other studies partially agree with those previously described that monensin use in lactating dairy cattle increased milk yield by 0.7 kg/year and improved milk production efficiency by 2.5%. Pinotti et al. (2007) found a significant positive effect of monensin supplementation on milk production of 2.9 kg/d for the first month of lactation. Similarly, Erdman and Sharma (1991) reported that monensin supplementation tended to increase milk yields in early lactation, while significantly improving milk production when supplemented in mid lactation. The effects of monensin supplementation consist of increased ruminal propionate production via changes in the rumen microorganisms (Hook et al. 2009), increased dry matter intake (Santos et al. 2019), nutrient digestibility and feed efficiency (Tseu et al. 2020), energy utilization (Tedeschi et al. 2003), milk yield in dairy cattle (Hausmann et al. 2017) and the expression of genes involved in the acid-base transport of the ruminal epithelium (Mirzaei-Alamouti et al. 2016). Monensin is a feed additive used to improve feed efficiency by depleting cells and selected microbes and altering rumen ecology (Bergen and Bates 1984). Monensin may also improve digestibility, absorption and retention of nutrients in cattle (Spears 1900), and has been extensively used to manipulate ruminal fermentation, and improve the performance and efficiency of the use of energy diet (Duffield et al. 2012). The mechanism of action to increase milk yield is that monensin increase the supply of glucogenic precursors resulting from changes in the pattern of rumen fermentation (Phipps et al. 2000). Cows treated with monensin

had a faster recovery of rumen functions after the typical impairment related to parturition. This improved capacity to overcome stressful conditions could account for the higher milk yield during the first 56 days of lactation (Mozzetti et al. 2019).

We noted that supplementation with monensin had an effect on milk LDH concentration. LDH levels in the milk on the 15th and 30th days of the study were 19.15% lower in the GK cows than in the GO group. Hiss et al. (2007) mentioned that LDH is generally accepted as a useful mastitis indicator. The use of monensin after calving reduced the incidence of clinical mastitis in cows. This effect reduced the rate of intramammary infection (by an approximate change in milk somatic cells to more than 250,000) in first lactation heifers by 13% (Duffield et al. 2012). Ionophores have a novel mode of action compared to other mastitis treatments and do not promote cross-resistance to classes of antimicrobials of medical significance (Russell and Houlihan 2003). Ionophores have in vitro activity against Gram-positive bacteria, including staphylococci, and oral administration of lasalocid and monensin in feed has reduced the incidence of mastitis in lactating dairy cows (Stefanska et al. 2015). As antiporters, ionophores prevent bacterial replication by disrupting concentration gradients of protons and metal ions across bacterial membranes (Russell and Houlihan 2003). Monensin and other ionophores have been shown to reduce the incidence of intramammary infection (McDougall et al. 2004) in dairy cows, and this is likely related to an improvement in the immune function due to better energy metabolism. Heuer et al. (2001) notice that cows were less predisposed to develop intramammary infections when treated with monensin before calving. The better utilization of endogenous energy sources, leading to a better immune response and increased energy metabolites, was related to immunosuppression (Ster et al. 2012). The mechanism suggested for this reduction is related to the better immune function of treated animals, caused by the improved energy (Duffield et al. 2012). Yasui et al. (2016) reported a tendency for a better function of neutrophils and monocytes in cows fed 450 mg/d of monensin during the transition period, suggesting a beneficial effect of the treatment on these leukocytes.

In cows which received supplementation with monensin BHB decreased from the beginning (0.04 mmol/L) of the experiment to 15-30 (0.06 mmol/L) days by 4.70%. According to the literature the use of controlled release monensin capsules significantly reduced the incidence of clinical ketosis post calving by 40% (Duffield et al. 2012). In the same study such antiketotic effects were also associated with changes in the rumen microflora and an increase in propionates. Propionate

is the major glucogenetic precursor taken up by the liver, with an estimated proportion of up to 32-73% of gluconeogenesis (Drong et al. 2016).

Monensin modulates the rumen microbiome, leading to more propionate production and providing more energy to the animal since propionate increases hepatic gluconeogenesis (Ipharraguerre and Clark 2003) and reduces the ketone body (Scharen et al. 2017) in blood circulation, indicating a reduction in lipolysis (Agustinho et al. 2021) The increase in ruminal propionate could be accompanied by a reduction in the amount of methane produced in the rumen and an increase in blood glucose concentration (Nagaraja et al. 1997). This study confirmed that monensin reduces BHB concentration in blood serum.

## Conclusions

The aim of this study was to compare the effect of controlled-release monensin on automatically registered BCS, and online biomarkers such as milk BHB, MY and LDH. We found that cows supplemented with monensin (a monensin controlled – release capsule) have lower risk of negative energy balance and subclinical ketosis (higher BCS at 15th and 30th days after calving, 4.70% decreased BHB from the beginning of the experiment to 15 and 30 days after calving and a steady increase in MY). We also confirmed that the cows which received a supplement with monensin had a lower risk of mastitis (lower LDH activity on the 15th and 30th days after calving).

Based on this case-control study, cows supplemented with a monensin capsule (21 days before calving) had a lower risk of NEB ( $p < 0.001$ ), ketosis ( $p < 0.001$ ) and mastitis ( $p < 0.001$ ).

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