

Monensin Controlled-release Capsules do not Change Performance and Metabolic Profile in Unchallenged Beef Cattle

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ABSTRACT

Background: Some additives are able to improve animal performance in growing and finishing periods. Monensin was first used to control coccidiosis in poultry and was extended to other animals, like ruminants, to act also as a growth promoter and improve cattle performance. In this species, monensin improves the synthesis of propionic acid in the rumen and decreases methane synthesis and protein degradation, resulting in better performance in protein and energy metabolism. The objective of this study was to evaluate the use of monensin controlled-release capsules on animals grazing *Lolium multiflorum* intercropped with *Trifolium repens* on metabolic profile and performance.

Materials, Methods & Results: Thirty Hereford cows were randomly distributed into two groups: control (CG) and monensin group (MG). Monensin was individually administered by controlled-release capsules placed in the rumen through oroesophageal pathway. All animals were identified through earring and kept under the same management condition, grazing on upland pasture mixture of *Trifolium repens* and *Lolium multiflorum*. Data from biochemical profile and performance were collected during 45 days. Blood samples started on the day of monensin controlled-release capsule placement (day 0) and continued in periods of 15, 30 and 45 days, after initial placement. Serum levels of albumin, glucose, urea, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were evaluated using colorimetric diagnostic kits. In the rumen fluid, pH was measured and protozoa count was performed. All statistical analyses were made using software SAS. Albumin, AST, glucose, LDH and urea were analyzed through MIXED procedure and Tukey-Kramer test was applied for comparison of means. For average daily gain, the orthogonal polynomials test was applied. Treatments did not differ in BSC, body weight and average daily gain (ADG). None of these performance parameters were significantly affected by the addition of monensin. Blood biomarkers did not show statistical differences between treatments and markers of rumen activity did not suffer interference from monensin supplementation. There was only a tendency ($P = 0.07$) for the first time (0) to a higher pH value in CG.

Discussion: Animals grazing in the finishing period, characterized by a continuous and linear weight gain, did not suffer any kind of stress situation. This condition did not provide a striking challenge that could reach the level of a metabolic change in animals. Facing feed shortages, or other stressful condition, supplementation with monensin and other additives, such as yeast, showed to be more effective, compared to animals in nutritional comfort. Weight gain increase is related to the expected changes in biochemical profile, as increased AST, glucose and LDH. The increase in AST levels on day 30 ($P < 0.0001$) is explained by the greater weight gain of animals in the previous period (day 15, $P < 0.0001$), where there was a higher hepatic activity to meet this anabolism and also by AST been an enzyme indicator of liver activity. This study did not show statistical treatment differences in relation to ruminal pH but, just a trend ($P = 0.07$) of higher pH in CG which is not caused by monensin supplementation that occurred since the first time (0), when animals were moved to pasture and receiving the monensin capsule. Since there was a low consumption of monensin capsules, the results were consistent with environment conditions and the phase in which the animals were. The results were also in agreement with finishing period, metabolic changes and animal performance at the same moment.

Keywords: production, blood biomarkers, ionophores.

INTRODUCTION

Feed have historically represented 50-70% of the cost of production in beef enterprises, demanding some effort to search for ways to improve animal efficiency. A feed efficiency of 10% across the entire feedlot sector would reduce feed costs about US\$ 1.2 billion. Some additives are able to improve animal performance in growing and finishing periods [18]. Monensin was first used for the control of coccidiosis in poultry and extended to other animals, like ruminants, to act also as a growth promoter and improve cattle performance by reducing or inhibiting the activity of ruminal microorganisms. More recently, monensin has been associated to environmental aspects, such as decreasing methane emission in cattle.

In Brazil, lasalocid and monensin are the only ionophores approved for use in ruminants feed [19]. In this species, monensin improves the synthesis of propionic acid in the rumen and decreases methane synthesis and protein degradation, resulting in better performance in protein and energy metabolism [7]. Positive effects on energy efficiency are the increasing in rumen propionate production, as a consequence of monensin resistance by the gram-negative bacteria, which reduce succinate to propionate, and a reduction in the gram-positive bacteria [16].

Therefore, the objective of this study was to evaluate the use of monensin controlled-release capsules on animals grazing *Lolium multiflorum* intercropped with *Trifolium repens* on metabolic profile and performance.

MATERIALS AND METHODS

The experiment was conducted on a farm in southern Brazil (located 33°41'28" S and 53°27'24" E). The experimental design was completely randomized with two treatments and considering each animal as an experimental unit. Thirty Hereford cows, with an average age of five years, body score condition (BSC) 3.6 ± 0.3 and average of body weight $382.7 \pm 2\text{kg}$, were randomly distributed in a control group (CG) and a monensin group (MG). Monensin was individually administered by controlled-release capsules (Rumensin Capsule)¹ placed in the rumen through oro-esophageal pathway supported by a specific applicator. The capsule used in this experiment contains ten tablets that release 320 mg of daily monensin in the rumen.

All animals were identified through ear tagging and kept under the same management condition, grazing on upland mixed pasture consisting of 27% of white clover (*Trifolium repens*), 48.37% of ryegrass (*Lolium multiflorum*), 19.63 % of other species not categorized during the experiment and 5% of species in senescence stadium. Samples of pasture were collected for feed analysis (Table 1).

Data from biochemical profile and performance were collected during 45 days. Blood samples started on the day of monensin controlled-release capsule administration (day 0) and continued in periods of 15, 30 and 45 days, after initial assignment. Blood samples were collected by puncture of the coccygeal vein and divided in EDTA (10%) and glycolytic pathway inhibitor (12% potassium fluoride) tube and one vial without anticoagulant. Immediately after collection, samples were centrifuged at 1800 g for 15 min and divided into two *Eppendorff* type tubes previously identified, one of which was frozen at -18°C and further cooled at 4°C.

Serum levels of albumin², glucose (Glucose Liquiform)², urea (Urea CE)², lactate dehydrogenase (LDH Liquiform)² and aspartate aminotransferase (AST/GOT Liquiform)² were evaluated using colorimetric diagnostic kits measured in a spectrophotometer (FEMTO 435, FEMTO®)³. Ruminal fluid collections were performed on days 0, 30 e 45 by oro-esophageal probe linked to a vacuum pump. The pH of rumen fluid, after being filtered through lint, was measured using a portable potentiometer (pHep4®, HANNA)⁴. Subsequently, the fluid was strained through gauze to obtain 5 mL samples for protozoa counting [15].

At the time of slaughter, on average 92 days after placement of the capsules, they were recovered and individual consumption of monensin each animal was measured.

Data analyses were performed using SAS statistical software⁵. Means were analyzed using the MIXED MODELS method. Comparison of means was performed using the Tukey-Kramer test ($P < 0.05$) were considered significant. For non-parametric data, analyses with chi-square and logistic regression were performed. The model used was:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + e_{ijk}$$

were, y_{ijk} is the dependent variable, μ the mean, α_i is the effect of the animal, β_j the period effect, τ_k is the treatment effect and e_{ijk} the residual

error. The adaptation to monensin inclusion was indirectly evaluated according periods using orthogonal polynomial contrasts [26] to determine linear and quadratic effects of the monensin consumption on average daily gain. Treatment effects for average daily

gain were tested for quadratic components through orthogonal polynomials. The period 1 refers to ADG between days 0 and 15 of the experiment, the period 2 between days 15 and 30 and the period 3 between days 30 and 45.

Table 1. Chemical composition of the diet during the experiment period.

Nutrient, %	Day 0	Day 15	Day 30	Day 45
Dry matter	15.9	16.2	24.4	23.5
Crude protein	16.3	17.2	14.8	15.7
Neutral Detergent Fiber	49.6	47.9	57.9	61.5
Acid Detergent Fiber	25.6	58.18	62.31	—
Crude Fat	2.4	2.4	—	2.5

RESULTS

No clinical signs of tympanism occurred. Consumption by animals fed monensin was 3.5 tablets per animal (1.120 mg of monensin) below the recommended by the manufacturer which suggests the consumption of 10 tablets in 100 days.

Treatments did not differ in BCS, body weight and average daily gain (ADG), which were not sig-

nificantly affected by the addition of monensin ($P > 0.10$) [Table 2].

Blood biomarkers did not show any differences between treatments (Table 3).

Monensin supplementation did not affect markers of rumen activity (Table 4). There was only a tendency ($P = 0.07$) during the first day (0) to a higher pH value in CG.

Table 2. Performance of cows supplemented with monensin controlled-release capsule (MG) and control group (CG).

Parameters	Day 0		Day 15		Day 30		Day 45		S.E.	<i>P</i> - Values		
	MG	CG	MG	CG	MG	CG	MG	CG		Group	Period	G*P
BSC	3.5 ^c	3.7 ^c	3.86 ^a	4.0 ^a	3.96 ^a	4.0 ^a	4.2 ^b	4.3 ^b	0.06	0.091	<0.0001	0.558
Body weight	382.6 ^a	382.8 ^a	396.0 ^{ab}	392.4 ^{ab}	422.5 ^{bc}	421.4 ^{bc}	445.9 ^c	442.2 ^c	14.3	0.911	<0.0001	0.830

Different letters in the same row indicate statistical difference ($P < 0.05$) by Tukey test between periods; BSC: Body Condition Score.

Table 3. Blood biomarkers of cows supplemented with monensin controlled-release capsule (MG) and control group (CG).

Biomarker	Day 0		Day 15		Day 30		Day 45		SE	<i>P</i> - values		
	MG	CG	MG	CG	MG	CG	MG	CG		Group	Period	G*P
Albumin (g/dL)	3.5	3.4	3.3	3.2	3.5	3.2	3.1	3.1	0.16	0.4676	0.2731	0.7185
AST* (U/L)	112.5 ^{ac}	114.9 ^{ac}	124.2 ^{ab}	119.7 ^{ab}	136.81 ^b	126.5 ^b	109.7 ^c	111.2 ^c	6.47	0.5564	0.007	0.7420
Glucose (mg/dL)	73.10 ^a	77.90 ^a	75.80 ^a	77.30 ^a	78.30 ^{ab}	78.20 ^{ab}	78.00 ^b	85.50 ^b	3.66	0.4294	0.0584	0.3988
LDH** (U/L)	428.6 ^a	464.9 ^a	465.6 ^{ac}	482.8 ^{ac}	491.8 ^{bc}	498.8 ^{bc}	535.6 ^{bc}	507.7 ^{bc}	27.36	0.7302	0.0336	0.6551
Urea (mg/dL)	42.4	46.50	42.3	44.3	45.9	46.7	41.2	40.4	4.04	0.5944	0.5947	0.9421

Different letters in the same row indicate statistical difference ($P < 0.05$) by Tukey test between periods; *Aspartate aminotransferase; **Lactate dehydrogenase.

Table 4. Ruminal biomarkers of cows supplemented with monensin controlled-release capsule (MG) and control group (CG).

Parameter	Day 0		Day 30		Day 45		S.E.	P - Values		
	MG	CG	MG	CG	MG	CG		Group	Period	G*P
pH	6.86 ^a	7.06 ^a	7.42 ^b	7.53 ^b	7.7 ^c	7.77 ^c	0.07	0.07	<0.0001	0.61
Protozoa (10 ³ /mL)	82	76	59	59	84	72	0.065	0.66	0.111	0.84

Different letters in the same row indicate statistical difference ($P < 0.05$) by Tukey test between periods.

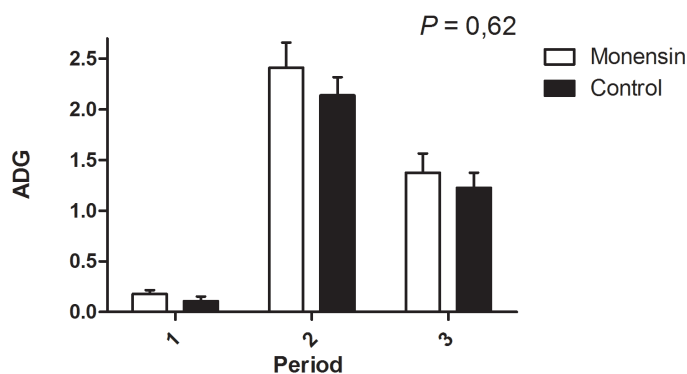


Figure 1. Graph representing the quadratic effect of gain weight in animals supplemented with monensin controlled-released capsules (GM) and control (GC). The period 1 refers to ADG between days 15 and 0 of the experiment, the period 2 between days 30 and 15 and the period 3 between days 45 and 30.

DISCUSSION

The beginning of the trial was at the same time of cattle grazing in a mixture of *Trifolium repens* and *Lolium multiflorum* pasture, which generally shows higher levels of crude protein and energy, and higher digestibility of ryegrass pasture compared to native grasslands of Rio Grande do Sul state [11], as shown in Table 1. Animals kept grazing in the finishing period, which is characterized by a continuous and linear weight gain, did not suffer any kind of stress situation, as shown in Figure 1. Thus, this condition did not provide a striking challenge that could reach the level of a metabolic change in animals. In a stressful condition, supplementation with monensin and other additives, such as yeast, showed to be more effective compared to animals in nutritional comfort [2], being in agreement with the results of the present trial.

Weight gain increase is related to the expected changes in biochemical profile, as increased AST, glucose and LDH. The increase in AST levels on day 30 ($P < 0.0001$) is explained by the greater weight gain of the animals in the previous period (day 15, $P < 0.0001$), where there was a higher hepatic activity to meet this anabolism and also by AST been an enzyme indicator of liver activity [10].

Albumin is considered the most sensitive metabolite for determining protein nutritional status. When albumin values are low, for a long period, it is possible to suggest an insufficient protein intake [22]. Serum albumin average was within the reference values established by [14]. It was suggest that a period of one month is required to detect significant changes in serum albumin concentration in ruminants due to the low rate of synthesis and protein degradation [21]. In this study, no significant difference in the levels of albumin were observed due to the short time between analyses.

Calving cows and ewes treated with monensin showed an increase in glucose levels, possibly due to an increasing of propionate in the rumen [8]. The finding that glucose concentration was higher in animals treated with monensin was not consistently reported. In the same way, glucose levels were similar throughout this study. Glucose concentrations did not differ between treatments ($P > 0.05$), probably due to the need for constant maintenance of this compound [6].

A number of studies in dairy cattle [9,12,23] reported an increase in blood, plasma or of urea serum concentration in animals treated with monensin. This effect reflects an increase in the flow or a non degradation of protein in the rumen to the intestine [8]. Paradoxically monensin is able to reduce ammonia

concentrations in the rumen by selectively inhibiting deamination bacteria [25]. Values of urea in the bovine plasma range from 17 to 45 mg/dL, keeping the animals in this study, both GM and GC, within the reference limits [14].

There was no difference between treatments in LDH levels, which remained within physiological parameters, and these results are in agreement with other study which observed that a low blood LDH can dismiss the risks of clinical acidosis [4]. The pH values suggest that animals were not in subclinical acidosis and, therefore, corroborate the LDH results. Decreased blood lactate dehydrogenase concentration reflects the reduced need to metabolize lactate from tissue metabolism and absorption from the digestive tract [20]. In the present study, LDH values are below those found by other authors [1].

In the same way, it was not possible detected any differences ($P > 0.05$) between treatments in relation to rumen pH, although a tendency ($P = 0.07$) of higher pH during the first period was observed in the CG group, the same moment when animals were moved to the pasture and receiving the monensin capsule, which could indicate a possible adaptation to the new diet. It was found an increase in rumen pH as a result of monensin utilization that possibly occurred due to a decrease on lactate producing bacteria, which could proliferate when monensin was not added to the diet [24]. Additionally, monensin would modulate rumen pH by controlling feed intake into more and smaller meals [17].

It is hypothesized that monensin does not affect methane production by inhibiting methanogens but instead inhibits the growth of bacteria, and protozoa, providing a substrate for methanogenesis [5]. The authors concluded that the effect of monensin on methane levels in the rumen is related to the ciliate protozoa

population and as this population adapted to monensin [13]. There was no difference between treatments in the total number of protozoa. The number of protozoa in the rumen fluid varies according the diet composition, time of feeding and the collection site in the rumen [3].

Benefits of monensin for feed utilization when cows are fed diets containing grain are not disputed, and no detrimental effects of monensin have been reported with cows fed forages as a sole diet [27]. Since there was a low consumption of monensin capsules, the results were consistent with environment conditions and the phase in which the animals were. The results were also in agreement with finishing period, metabolic changes and animal performances at the same moment.

CONCLUSION

In conclusion, there is no influence of monensin addition in finishing cows grazing *Lolium multiflorum* intercropped with *Trifolium repens* on performance and biochemical parameters, as they were not enough challenged since they were receiving sufficient amounts of nutrients during this period, implying in a low monensin consumption.

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Ethical approval. This research project was approved by the Ethics Committee for Animal Experimentation of UFPEL, under protocol number 3039/2014, in accordance to Brazilian Laws and ethical principles published by the Brazilian College of Animal Experimentation.

Declaration of interest. The authors report no conflicts of interest, and they alone are responsible for the content and writing of the paper.

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