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Probiotics Improve Productive Performance and Carcass Ultrasonographic Quality of Steers under Grazing during Dry-Water Transition Season

Research Article

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ABSTRACT

The aim of this study was to evaluate the probiotic effect on productive performance and carcass ultrasonographic quality of cross-bred steers. Ninety-four cross-breed steers with initial BW of 208.53 ± 23.56 kg were used, and distributed in a completely randomized design composed of 2 nutritional treatments, monensin and probiotic. The animals were weighed every 30 days, evaluated for productive performance and submitted to ultrasound evaluation of the carcass at the end of the experiment to verify development of the longissimus muscle area (LMA), subcutaneous back fat thickness in the longissimus dorsi muscle. The daily weight gain was greater in the group that received probiotics. Feed intake did not differ between groups. The animals that received probiotics obtained LMA and back fat thickness greater than the treatment with monensin. It can be concluded that the probiotic used in this trial provides improvements in the productive performance, helps in the digestibility of the fiber and improves the evaluated carcass characteristics.

KEY WORDS antibiotics free, carcass quality, clean production, ionophores.

INTRODUCTION

Brazilian beef cattle have been going through an important transition period given the need for better efficiency in production systems, quality of the final product, that is, better quality of the meat produced. However, all these particularities must be in accordance with sustainable production practices in order to serve foreign markets (Santos, 2009). The efficiency of the production systems is linked to the seasonal nature of the production of tropical forages, nutritional plans with low intake of supplements, thus the search for sustainable nutritional efficiency has promising prominence within the grazing systems (Euclides, 2007). Due to the need to improve productive efficiency in livestock and optimize rumen fermentation (Mccann *et al.* 2017), additives in diets have become a great tool, with ionophores being the most used. Ionophores are antimicrobial compounds used to improve feed efficiency in ruminants, due to changes in the ruminal and intestinal microbiota, less methane production and consequently reducing energy losses during fermentation. The most common of these are: monensin, lasalocide, laylomycin and salinomycin (Vohra *et al.* 2016).

However, the use of antimicrobials in animal feed has been the target of criticism, due to the accumulated residues in products for human consumption and risk of antimicrobial resistance (Wegerner, 2003). In this way, the European Union Since 2006 has banned the use of antibiotics as promoters of animal growth, as well as the import of animal products from countries that use them. Since then, strate-

gies such as organic acids, probiotics, saponins, tannins and essential oils have been studied (Morais, 2011).

Probiotics are used in food in the form of combinations of bacteria and / or yeasts, to promote balance of microbial flora, manipulating rumen fermentation and seeking to improve fermentative efficiency by increasing productivity (Chaucheyras-Durand, 2012). Several lactic acid bacteria (LAB) strains species belonging to the genera *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*, are considered beneficial to the host and have, thus, been used as probiotics and included in several functional foods (Uyeno *et al*. 2015).

Probiotics have the ability to enhance intestinal health by stimulating the development of a healthy microbiota (predominated by beneficial bacteria), preventing enteric pathogens from colonizing the intestine, increasing digestive capacity, lowering the pH, and improving mucosal immunity.

It is important for the introduced microbes not to disturb the indigenous population, which has already been adapted to the environment of the gut tract to work both for and with the host (Frizzo *et al*. 2011). Kelsey and Colpoys (2018), supplementing beef cattle with 10 g of lactic acid bacteria per day for 90 days observed greater weight gain and feed conversion for the group that received probiotic. Thus, the objective of the present research was to evaluate the effect of probiotics on animal performance and carcass characteristics of crossbred steers under grazing during dry-water transition season.

MATERIALS AND METHODS

The experiment was carried out on a commercial farm in the municipality of Angélica, Mato Grosso do Sul, between October 2017 and February 2018, totaling 120 days. Ninety four crossbred steers with an initial BW of 208.53 ± 23.56 kg were used, which were in rotational grazing of *Brachiaria brizantha* cv. brspiatã, with protein-energetic supplement with 200 g kg^{-1} of crude protein and 625 g kg^{-1} of total digestible nutrient, expected intake of 2 g/100 kg of BW. To supply the animals, additives were incorporated into the supplements to facilitate their homogenization.

Ninety-four animals were used, distributed in two groups of forty-seven animals .The animals were distributed in a completely randomized design comprising 2 groups: 1) monensin, supplemented with 300 mg/day of sodium monensin; 2) probiotic**,** supplemented with 1g kg^{-1} for every 100 kg of BW (*Bacillus subtilis* 3.0×10^9 UFC g^{1}, *Bifidobacterium bifidum* 1.0×10^9 UFC g^{-1}, *Enterococcus faecium* 1.0×10^9 UFC g^{-1}, *Lactobacillus acidophilus* 1.0×10^9 UFC g^{-1}, *Lactobacillus buchneri* 2.0×10^9 UFC g^{-1}, *Lactobacillus casei* 1.0×10^9 UFC g^{-1}, *Lactobacillus lactis* 1.0×10^9 UFC g^{-1}, *Saccharomyces cerevisiae* 2.0×10^8 UFC g^{-1} Biomart Animal Nutrition, Brazil). Protein-energy supplements were formulated according to Valadares Filho *et al*. (2016) for weight gain of approximately 800 g/d.

The forage samples were collected randomly at 50 points per paddock, with the aid of a square with an area of 0.25 m^2, cut at grazing height (30 cm). The production of forage (fresh matter/ha and DM/ha) was carried out according to the methodology described (Euclides, 2007) (Figure 1). To determine the botanical composition, the samples were subsampled. The material was separated manually in leaf blade and stem + sheath (Almeida *et al*. 2003).

Afterwards, all samples were submitted to drying in an oven with forced air ventilation for 72 hours at 55 ˚C, with the dry material crushed in a cyclone mill. All samples obtained were submitted to laboratory procedures and the contents of dry matter (DM) and crude protein (PB) were evaluated according to (AOAC, 2000) and neutral detergent fiber (NDF), acid detergent fiber (ADF), as described by Van Soest *et al*. (1991).

Animals were weighed after 12 h of feed restriction, on arrival, at 0, 30, 60, 90, and 120 d. The Average daily gain (ADG) was calculated as the slope of the linear regression of the weights over the experimental period.

Supplement intake was measured daily in the morning. The orts of the experimental supplements remained between 5 and 10% ensured *ad libtium* intake. Ultrasound measurements were taken at the beginning and the end of the feeding period using an Aloka® model SSD 500 Micrus (Aloka Co. Ltd., Zug, Switzerland) with a linear probe (3.5 mHz, 172 mm in length). Ultrasound measurements were taken between the 12^{th} and 13^{th} ribs to determine the longissimus muscle area (LMA) and backfat thickness. In the ultrasonic evaluation, the animals were immobilized in an individual trunk with a triple containment system, using guillotines, and the measurement site was covered with a thin layer of oil, immediately before taking images in the region between the 12^{th} and 13^{th} ribs, in order to guarantee maximum resolution, through the acoustic contact of the probe's standoff with the animal's skin.

Statistical analysis

All statistical analyses were conducted using SAS software (SAS, 2004). Firstly, the normality of residuals and the homogeneity of variances were verified using the univariate procedure of SAS software. The initial weight was used as a co-variable but was removed from the model because it was not significant.

Productive performance data were analysed according to the following model:

$Y_{ijk} = \mu + T_i + a_{j:i} + M_k + T \times M_{ik} + e_{ijk}$

with $a_{j:i} \approx N(0, \sigma_a^2)$ and $e_{ijk} \approx MVN(0, R)$

Where:
Y_{ijk}: dependent variable.
μ: overall mean.
T_i: fixed effect of treatment (i=1 and 2).
$a_{j:i}$: random effect of animal j within i treatment (j=1 to 94).
M_j: fixed effect of time (k=1 to 5).
$T \times M_{ik}$: fixed effect of the interaction between treatment and time of evaluation.
e_{ijk}: residual error.
N: Gaussian distribution.
σ^2_a: variance associated with the random effect of the animals.
MVN: multivariate normal distribution.
R: matrix of variance and covariance due to repeated measures.

Akaike's method was used to choose the *R* for each variable. The AR(1) was used in de model. Significance was defined at $P \leq 0.05$.

Carcass ultrasonography were analysed according to the model:

$Y_{ij} = \mu + T_i + a_{j:i} + e_{ij}$

Where:
Y_{ijk}: dependent variable.
μ: overall mean.
T_i: fixed effect of treatment (i=1 and 2).
$a_{j:i}$: random effect of animal j within i treatment (j=1 to 94).
e_{ij}: residual error.

Significance was defined at $P \leq 0.05$.

RESULTS AND DISCUSSION

The content of NDF, ADF and CP of pastures had no influence on the performance of experimental groups, which was expected because the animals were on the same type of pasture and rotated between the paddocks, indicating that the difference in weight gain between the groups was due to the treatment. However, it can be seen that the group that received probiotic obtained a superior result in weight gain and there was no difference in supplement intake, suggesting that the probiotic altered the dynamics of rumen fermentation and fiber utilization, selected fibrolytic bacteria, increasing the digestibility of the fiber by increasing the concentrations of acetate, butyrate and proportionally that of propionate (Frizzo *et al.* 2011). Time had a positive interaction on treatments (Table 1).

The highest ADG for the group that received probiotic supplementation compared to the group that received monensin, is in accordance with results compiled by Vohra *et al.* (2016), demonstrating the effects of probiotics in the feeding of ruminants, which observed an increase in fiber digestion and concluded that one of the main mechanisms by which this is obtained is through the growth of the community of fibrolytic bacteria and by the increase of the activities of fermentation of cellulolytic bacteria, such as *Fibrobacter succinogenes* and *Ruminococcus albus*.

Krehbiel *et al.* (2003) summarized data from experiments that used probiotics to feed cattle, containing bacteria of the genus *Lactobacillus* ssp., indicating that such additives improve feed efficiency and ADG, being able to increase the level of ruminal propionate, improve energy efficiency and, therefore, assist in animal performance.

Ribeiro *et al.* (2015) compared the performance of crossbred heifers that received monensin sodium and symbiotics and can conclude that the group that received the symbiotic did not differ statistically from treatment with monensin for ADG and feed efficiency. These data corroborate the use of probiotics and / or symbiotics in improving animal performance and replacing ionophore antibiotics.

It can be seen that in the first 60 days of supplementation (Figure 2), monensin promoted superior results in weight gain, and after 60 days the group that received probiotic gained significantly more weight. These data indicate that the probiotic needs a longer adaptation period for its best efficiency, while monensin in the short term improved the rates, but in the medium to long term there is evidence that the rumen microorganisms adapt and no longer have a significant effect on weight gain, data that were also observed by Melchior *et al.* (2018) and in the study by Guan *et al.* (2006), which the authors observed that after 6 weeks of supplementation of monensin for Angus steers, the population of microorganisms was restructured, indicating their adaptation to the compound.

For the longissimus area muscle (LAM) measurement, the group that received probiotic additives in the diet was statistically superior to the one that received monensin ($P<0.05$), the same occurred for the measurement of subcutaneous fat thickness (Table 2). There was no interaction between LAM and backfat thickness data. The variable LAM/100 kg body weight (BW) was higher in the group that received probiotics, while backfat thickness/100 kg had no difference between treatments. Supplement intake did not differ between groups, with the result of increased rib eye area and subcutaneous fat being caused by the addition of probiotic in the diet, a result consistent with greater weight gain in the same group.

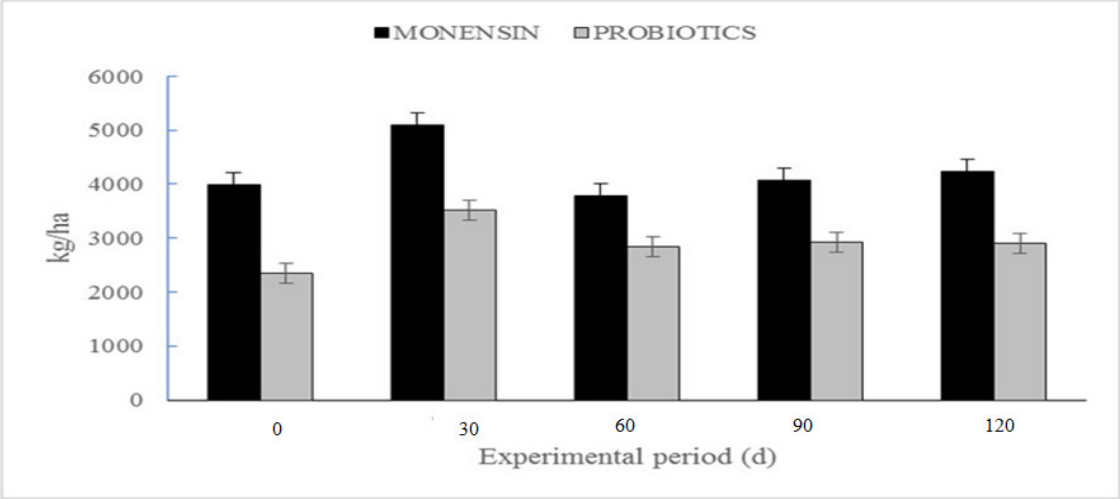

Figure 1 Forage dry matter availability (kg/ha) over the experimental period
Supplement P ≤ 0.001; Time P ≤ 0.002 and Interaction P ≤ 0.023

Table 1 Productive performance, availability and nutritional value of forage according to experimental supplements

Item	Experimental supplements[1]		SEM	P-value		
	Monensin	Probiotics		Supplement	Time	Interaction
kg/d						
Averagedailygain	0.895	0.963	0.34	0.043	0.001	0.022
Intake (% BW)						
Supplement	0.265	0.293	0.02	0.432	0.542	0.762
Forage availability (kg)						
Freshmatter	13670	10038	3.45	0.001	0.003	0.034
Drymatter	4302	2963	2.78	0.001	0.002	0.023
Chemical composition (% DM)						
Whole plant						
Drymatter	31.47	29.51	2.34	0.023	0.001	0.023
Neutral detergente fiber	58.45	57.76	1.58	0.543	0.003	0.043
Acid detergente fiber	28.96	28.89	1.18	0.656	0.023	0.432
Crudeprotein	10.17	10.09	0.82	0.545	0.032	0.003
Leaf						
Drymatter	35.78	40.09	3.56	0.032	0.001	0.653
Neutral detergente fiber	55.84	49.91	3.09	0.012	0.043	0.234
Acid detergente fiber	30.03	32.33	2.67	0.432	0.032	0.651
Crudeprotein	13.85	13.60	1.14	0.327	0.022	0.665
Culm						
Drymatter	28.29	37.23	2.98	0.012	0.001	0.332
Neutral detergente fiber	62.62	61.85	3.65	0.652	0.004	0.443
Acid detergente fiber	32.87	33.37	2.09	0.688	0.012	0.551
Crudeprotein	9.95	9.35	1.14	0.384	0.032	0.540

[1] Monensin (300 mg/animal/d); probiotics (1 g/100 kg BW *Bacillus subtilis* 3.0×10^9 UFC g^{-1}, *Bifidobacterium bifidum* 1.0×10^9 UFC g^{-1}, *Enterococcus faecium* 1.0×10^9 UFC g^{-1}, *Lactobacillus acidophilus* 1.0×10^9 UFC g^{-1}, *Lactobacillus buchneri* 2.0×10^9 UFC g^{-1}, *Lactobacillus casei* 1.0×10^9 UFC g^{-1}, *Lactobacillus lactis* 1.0×10^9 UFC g^{-1} and *Saccharomyces cerevisiae* 2.0×10^8 UFC g^{-1}).
SEM: standard error of the means.

In similar studies, some authors compared the performance of carcass characteristics of cattle that received probiotics and monensin in supplementation, however, there was no difference in LAM and backfat thickness variables between treatments (Gomes *et al*. 2009; Kuss *et al*. 2009; Rigobelo, 2014; Ribeiro *et al*. 2015).

However, these authors worked with different probiotic formulations, concluding that the effects are directly linked to the type of microorganisms offered, interaction with the type of diet as well as the characteristics of the animals.

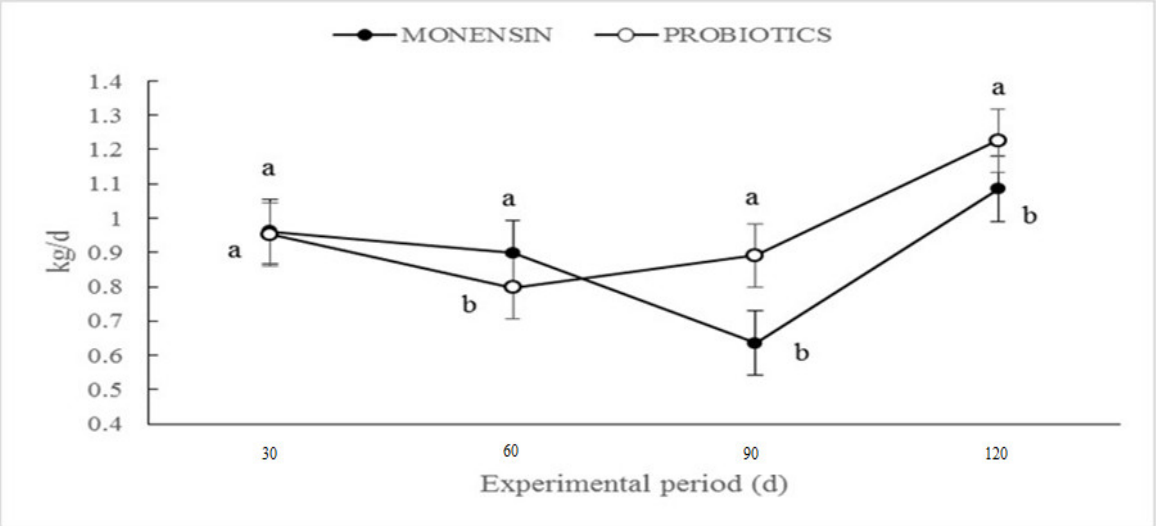

Figure 2 Average daily gain (ADG) (kg/d) over the experimental period
Supplement P= 0.043; Time P= 0.001 and Interaction P= 0.022

Table 2 Carcass ultrasound measurements according to experimental supplements

Item	Experimental supplements[1]		SEM	P-value
	Monensin	Probiotics		Supplement
Longissimus muscle area (cm^2)	38.83	40.14	1.10	0.012
Backfat thickness (mm)	5.24	5.33	0.02	0.043
Ratio	0.538	0.552	0.01	0.127
Longissimus muscle area/100 kg BW	14.06	15.09	0.47	0.032
Backfat thickness/100 kg BW	1.87	1.89	0.04	0.542

[1] Monensin (300 mg/animal/d); probiotics (1 g/100 kg BW *Bacillus subtilis* 3.0×10^9 UFC g^{-1}, *Bifidobacterium bifidum* 1.0×10^9 UFC g^{-1}, *Enterococcus faecium* 1.0×10^9 UFC g^{-1}, *Lactobacillus acidophilus* 1.0×10^9 UFC g^{-1}, *Lactobacillus buchneri* 2.0×10^9 UFC g^{-1}, *Lactobacillus casei* 1.0×10^9 UFC g^{-1}, *Lactobacillus lactis* 1.0×10^9 UFC g^{-1} and *Saccharomyces cerevisiae* 2.0×10^8 UFC g^{-1}).
SEM: standard error of the means.

In another study, carried out by Baah *et al.* (2009), with probiotics based on *Lactobacillus casei* and *Lactobacillus lactis*, the authors reported an improvement in feed efficiency and average daily gain, however there was no effect of including this additive on carcass performance, concluding that despite improving fermentation, it is not guaranteed improvement in carcass characteristics, suggesting that differences in intestinal microbial ecology varying according to diet, would be responsible for this result found.

Probiotics have the ability to modify the profile of ruminal and intestinal fermentation, in rumen by selection of fibrolytic bacteria and in some cases amylolytic, which can increase the concentration of short-chain fatty acids, in the intestine the strains beneficial from bacteria acidify the environment and increase the absorption of nutrients which contributed to improve the carcass quality of supplemented animals (Kelsey and Colpoys, 2018).

The results found suggest that the probiotic formulation used in this assay provided an improvement in carcass characteristics, mainly due to the increase in longissimus muscle area, which is directly related to the cut yield (Hedrick, 1983). It is worth mentioning the known effects of monensin (Duffield *et al.* 2012) on improving food efficiency by decreasing intake and increasing average daily gain, thus, the possibility of using an additive with similar or superior effects, like the one used in this study, it is interesting when you want to reduce the use of antibiotics in animal feed.

CONCLUSION

The use of probiotics in energy protein supplements for cross-bred steers under grazing positively influenced the average daily weight gain and carcass ultrasonography quality, showing that it can be used in clean production systems insteadof antibiotics as growth promoters.

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