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Virginiamycin and sodium monensin supplementation for beef cattle on pasture

[*Suplementação com virginiamicina e monensina sódica para bovinos de corte a pasto*]

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ABSTRACT

The aim of this paper was to evaluate the effects of including virginiamycin (VM), sodium monensin (MON) or the association (VM+MON) in the energetic mineral supplement, on the intake and performance of beef cattle on pasture. Forty Nelore heifers with 24 months of age and initial body weight (BW) of 251.5±16.6kg, were distributed in four treatments in a randomized block design. Treatments consisted of adding VM, MON or VM+MON to the supplement (CONT). Additive concentrations were defined to reach a dose of 40 to 45mg/100kg BW. The herbage allowance was not a limiting factor for the animals' intake. Supplement intake was lower than expected, with 33.0, 18.8 and 26.3mg per 100kg BW for VM, MON and VM+MON, respectively. Dry matter intake (DMI, mean=2.65% BW) and animal performance were not affected by the inclusion of additives. The average daily gain (ADG) was 0.561kg/animal day⁻¹. The inclusion of additives in energetic mineral supplement does not affect the DMI and the ADG of grazing animals. The variability in supplement intake and daily dose intake of additives may have influenced the performance of the animals. Monensin inclusion presented the less expensive supplementation cost, due to reduction in supplement intake without changing weight gain.

keyword: additives, heifers, intake, ionophores, performance

RESUMO

Objetivou-se avaliar o efeito da inclusão dos aditivos virginiamicina (VM), monensina sódica (MON) e associação (VM+MON) no suplemento energético-mineral sobre o consumo e desempenho de bovinos manejados em pastagem de Urochloa Brizantha cv. Marandu. Foram utilizadas 40 novilhas Nelore com 24 meses de idade e peso corporal (PC) inicial médio de 251,5±16,6kg distribuídas em delineamento em blocos ao acaso com quatro tratamentos. Os tratamentos consistiam de suplemento energético-mineral (CONT) acrescido de VM, MON ou VM+MON. A oferta de forragem não limitou o consumo dos animais. O consumo dos aditivos foi de 0; 33,0; 18,8 e 26,3mg por 100kg de PC para CONT, VM, MON e VM+MON, respectivamente. O consumo de matéria seca e o desempenho dos animais não foram afetados pela inclusão dos aditivos. O ganho médio diário dos animais foi de 0,561kg dia⁻¹, sem diferença entre os tratamentos. A inclusão de aditivos no suplemento energético-mineral não alterou o CMS e o desempenho dos animais em pastejo. A variabilidade no consumo de suplemento e na dose ingerida dos aditivos pode ter influenciado o desempenho dos animais.

Palavras-chave: aditivos, consumo, desempenho, ionóforo, novilhas

INTRODUCTION

The use of technologies to increase the feed efficiency and weight gain of animals are increasingly recommended for grazing systems, due to the fact that about 90% of the Brazilian bovine herd is exclusively fed on pasture (Silva *et al.*, 2016). The anti-microbial growth promoters, such as antibiotics ionophores or non-ionophores, are alternatives to improve the efficiency of nutrient utilization, since they can manipulate ruminal fermentation through changes in the microbial population (Tedeschi *et al.*, 2011). In grazing animals, these alterations in pattern of rumen fermentation promoted by additives can reduce methanogenesis, a strategy to mitigate greenhouse gases (Guan *et al.*, 2006), suppling the increasing demands for sustainable livestock production (Godfray *et al.*, 2010; Garnett *et al.*, 2013).

Sodium monensin is the most studied and widely used growth promoter in the world. Mainly because monensin could improve energetic efficiency in the cattle through increased propionic acid production and a reduction in methane production (Erasmus *et al.*, 2008; Neumann *et al.*, 2018). Although the use of virginiamycin as growth promoter for grazing animals is recent, studies have shown that their inclusion in supplements may increase weight gain in grazing animals and improve the efficiency of supplementation programs through increased utilization of nutrients modulating ruminal fermentation (Salinas-Chavira *et al.*, 2009; Nuñez *et al.*, 2013; Beck *et al.*, 2014). The use of monensin and virginiamycin association in

beef cattle supplements, to our knowledge, is not yet fully characterized; however, this combination has the potential to maximize nutrient absorption in the rumen and increase animal performance.

One of the challenges in using growth promoters in grazing systems is to feed the animals and ensure the frequency and amount of daily intake. The efficiency in ruminal microbial selection depends on the continuous consumption of additives, which could be limited due to variations in voluntary intake of supplements (Cockwill *et al.*, 2000). The difficulty in reaching the desired consumption by the herd could also contribute to the lack of positive results (Bowman and Sowell, 1997). The objectives of the present study were to evaluate the effects of the inclusion of virginiamycin and/or sodium monensin into the energetic mineral supplement, on intake and performance of beef heifers on pastures of Marandu grass. Our hypothesis is that the inclusion of additives into the energetic mineral supplement will increase the performance of grazing beef heifers and will reduce production costs.

MATERIAL AND METHODS

The Animal Ethics Committee of the Federal University of Minas Gerais (protocol n. 12/2013) approved the protocols for this study. The study was conducted in an experimental farm, located in Igarapé county, Minas Gerais state, Brazil (20°4'12"S, 44°18'7"W), between February 1 and June 30, 2014. Precipitation and temperature were measured daily (Figure 1).

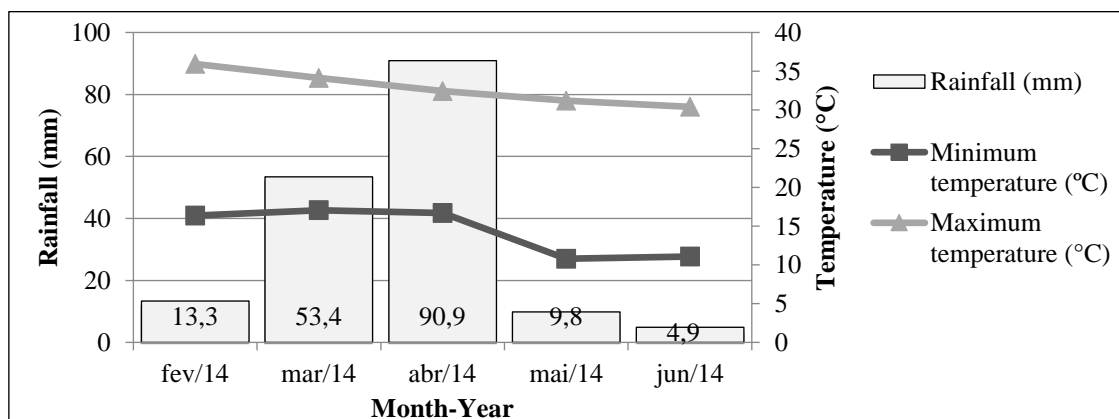


Figure 1. Climate data for the experimental period from February to June 2014, measured at the farm.

The experimental area consisted of 12 *Urochloa brizantha* cv. Marandu (syn. *Brachiaria brizantha*) grass paddocks, of two hectares (ha) each, provided with drinkers and mineral supplement feeders. Pasture was fertilized in March 2014, with 45kg of nitrogen per ha. Forty Nellore heifers (251.5±16.6kg of live weight, 24 months of age) were used. Animals were distributed randomly in a block design to one of the four treatments: CONT- energetic mineral

supplement (control treatment); VM – energetic mineral supplement with virginiamycin; MON – energetic mineral supplement with sodium monensin; VM+MON – energetic mineral supplement with virginiamycin and sodium monensin (Table 1). Supplements were mixed by the PRODAP company (Belo Horizonte, MG, BR) using virginiamycin 50% (Phibro, Ridgefield Park, NJ, USA) and sodium monensin 10% (Elanco, Greenfield, IN, USA).

Table 1. Ingredients and chemical composition of the energetic mineral supplements fed to the grazing Nellore heifers during the experimental period (in g/kg dry matter)¹

	CONT	Virginiamycin (VM)	Monensin (MON)	VM+MON
Ingredients, g/kg DM				
Ground corn	555.6	555.6	555.6	555.6
Sodium chloride	173.6	173.6	173.6	173.6
Mineral premix	92.6	92.6	92.6	92.6
Dicalcium phosphate	89.3	89.3	89.3	89.3
Kaolin	88.9	88.3	85.6	85.0
Monensin 10%	-	-	3.35	3.35
Virginiamycin 50%	-	0.63	-	0.63
Chemical composition				
Dry matter	897.2	902.9	902.1	901.6
Crude Protein	458	507	489	501
TDN ²	474.0	453.6	451.3	469.4
Ether extract	15.5	14.3	16.3	17.0
Cost, R\$/kg of supplement	1.21	1.55	1.29	1.63

¹ CONT: energetic mineral supplement without additives (control); VM – energetic mineral supplement with virginiamycin; MON – energetic mineral supplement with sodium monensin; VM+MON – energetic mineral supplement with virginiamycin and sodium monensin. Amounts of minerals (per kg of supplement): 48.03g Ca; 14.4g P; 3.67g S; 3.36g K; 1.89g Mg; 1679mg Zn; 1352mg Fe; 591.9mg Cu; 287.8mg Mn. ² Total digestible nutrients calculated according NRC (Nutrient..., 1996) (4.409kcal equates to 1g of TDN).

Ten animals were allocated in each one of the four treatments and divided in three blocks (four paddocks each). One of the blocks with 16 animals (four animals per treatment) and the others with 12 animals (three animals per treatment). Continuous grazing system was used, although the animals were moved in a 7-day period into another paddock aiming for the elimination of possible paddock effects. Animals went through a 45 day pre experimental period, in which they received the control supplement (without additives) in order to adapt to the experimental management and to measure the supplement intake. Additive concentrations were defined in order to reach a dose of 40 to 45mg per 100kg of body weight (BW). Supplements were fed *ad libitum* in feeders and daily consumption was calculated dividing the amount consumed weekly by the number of animals in each paddock.

The forage dry matter (DM) availability was performed every 28 days by cutting 5 randomly selected spots (1.0m × 1.0m) to ground level (5cm stubble height) using hand shears as described by McMeniman (1997). All mown herbage was collected, weighed and subsampled for analysis of potentially digestible DM (pdDM) and for plant components ratio (leaf, stem, senescent material). The pdDM was calculated with the following equation: pdDM= 0.98 x (100 - NDF) + (NDF - iNDF), in which NDF = neutral detergent fiber and iNDF = indigestible neutral detergent fiber. The iNDF was obtained after *in situ* incubation of a whole plant sample for 288 hours in the rumen of a cannulated bovine (Valadares Filho *et al.*, 2010). To calculate herbage allowance, the data of forage production (kg DM/day) in function of the animal's body weight (kg) was used. Along with forage sample, a graze simulation of each paddock was

performed every 28 days, as described by Euclides *et al.* (1992).

The samples from the simulated grazing were dried in a forced air ventilation oven at 55°C for 72 hours and ground through a 1mm screen in a Willey mill (Alpax, Diadema, SP, BR). DM levels were determined by the 934.01 method, crude protein (CP) by the 984.13 method, neutral detergent insoluble nitrogen (NDIN) by the 984.13 method, ether extract (EE) by the 920.85 method and ash by the 938.08 method, according to the AOAC (Official..., 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin analysis were performed through the sequential method of Van Soest *et al.* (1991). Calcium and phosphorus levels were determined using titration and colorimetric method, respectively. Total digestible nutrients (TDN) were calculated using the equation: $TDN = 83.39 - (0.4171 \times NDF)$ (Capelle *et al.*, 2001) and the non-fiber carbohydrates (NFC) using the following equation: $NFC = 100 - (\%CP + \%NDF + \%EE + \%Ash)$ (Sniffen *et al.*, 1992). To determine individual DMI the following formula was used: $DMI (g/day) = FP / (1 - (IVDMD/100))$; in which FP = fecal production and IVDMD = *in vitro* dry matter digestibility.

The FP was obtained using the external marker Lipe® (Produtos de Pesquisas Simões Saliba, Florestal, MG, BR), that was provided in May to 16 animals, four of each group, from the same block, in a completely randomized design. Lipe® was supplied in a capsule with 0.5g/animal day⁻¹ for seven days, two days of adaptation and five days of feces sampling. Fecal samples were dried in forced air ventilation oven at 55°C for 72 hours and ground through a 1mm screen in a Willey mill (Alpax, Diadema, SP, BR), and then homogenized to produce a composed sample for each animal. The Lipe® concentrations in the feces were measured using Near Infrared Spectroscopy (Femto, modelo NIR 900 PLS, SP, BR), by the logarithmic ratio of absorption intensities from spectral bands (Saliba *et al.*, 2015). FP was obtained by the formula: $FP = Lipe® \text{ administrated } (g/day) / \text{feces Lipe® concentrations } (g/kg \text{ of DM})$.

To determine IVDMD, simulated grazing samples from the month of May were weighted

in F57/ANKOM bags (Ankon Technology Corp., Fairport, NY, USA) and incubated in fermentation bottles. The inoculum was prepared with ruminal fluid of cannulated cows, previously fed with the experimental energetic mineral supplement. To do this, a square Latin 4x4 design was used, with four cannulated cows and four supplements. Ruminal fluid from cannulated animals was collected 15 days after the adaptation to the supplement, in every experimental period. After 96 hours of incubation, the bags containing the degradation residue were washed and dried in oven at 105°C for 12 hours. The IVDMD was calculated by the difference between the incubated feed and the residue after incubation.

Average daily gain (ADG) was obtained by the difference between the final weight and the initial weight divided by the experimental period. The feed efficiency was calculated by the ratio between the ADG and the estimated DMI during the measuring period. To evaluate the animal performance analysis a randomized block design was used, and to evaluate the forage intake and feed efficiency a completely randomized design was used. The statistical analyses were performed using GLM procedure from the statistical analyses system (SAS 8.0), and the averages were compared by the Tukey's test, with 5% significance. The supplement intake data was submitted to polynomial linear, quadratic and cubic regression analysis and the graphs created in the excel program (Microsoft Corporation, Redmond, WA).

RESULTS AND DISCUSSION

The total dry matter (TDM), potentially digestible DM (pdDM) and green leaf and stem DM (LSDM) availability presented average values of 3.3; 2.3 and 1.4 tons of DM/ha, respectively. Hbage allowance (HA) in LSDM was greater than 11% in all the evaluated months (Figure 2). The HA between 10 and 12% of the BW in TDM does not limit the animal's intake and makes possible the maximum pasture DMI (Hodgson, 1990). Therefore, the higher leaves and stem availability suggest that animals were able to select the plant components with better nutritional value.

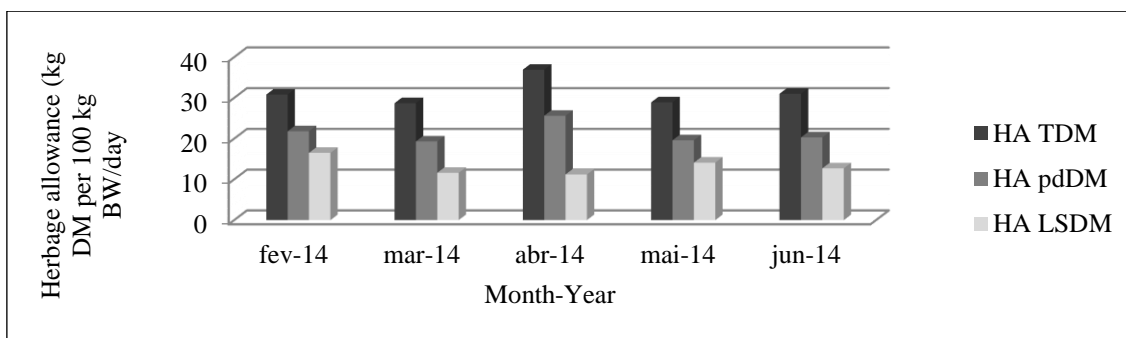


Figure 2. Herbage allowance (HA) in total dry matter (HA TDM), in potentially digestible DM (HA pdDM) and in green leaf and steam DM (HA LSDM) measured during the experimental period.

The simulated graze samples composed mainly by leaves represent the quality of the forage consumed by the animals, since the LSDM availability was not a limiting factor. The pasture nutritional composition presented increased crude protein (CP) content during the experimental period (Table 2), although the relation TDN:CP presented an average of 5.43, below the limit (7.0), in which we would not expect reductions in the voluntary forage intake (Moore *et al.*, 1999). The accumulated rainfall in March and April (144.3mm, Figure 1) and pasture fertilization could explain the higher CP content, causing a smaller decrease in the TDN:PB when compared to the other months. When these components ratio is unbalanced, there is a limitation and a deficiency in the use of microbial protein in the rumen, compromising animal performance (Detmann *et al.*, 2014).

During the rainy season, pasture usually exhibits a relative excess of protein in relation to energy levels, especially if fertilization is used, as observed in the present study (Table 2). Therefore, the inclusion of corn as an energy source in the supplement provided to the animals was necessary, aiming to increase the available energy and leading to a better TND: PB ratio in the diet.

Average voluntary supplement intake during the experimental period was 0.289kg/animal, day⁻¹ for the CONT, 0.262kg/animal, day⁻¹ for the VM, 0.157kg/animal, day⁻¹ for the MON and 0.194kg/animal, day⁻¹ for the VM+MON treatments. The expected supplement intake (400g/animal day⁻¹) was not reached during the experimental period, resulting in an intake of additives of 33.5; 18.8 and 26.3mg per 100kg PC of VM, MON and VM + MON, respectively.

Table 2. Chemical-bromatological composition of *Urochloa brizantha* cv. Marandu grass during the experimental period

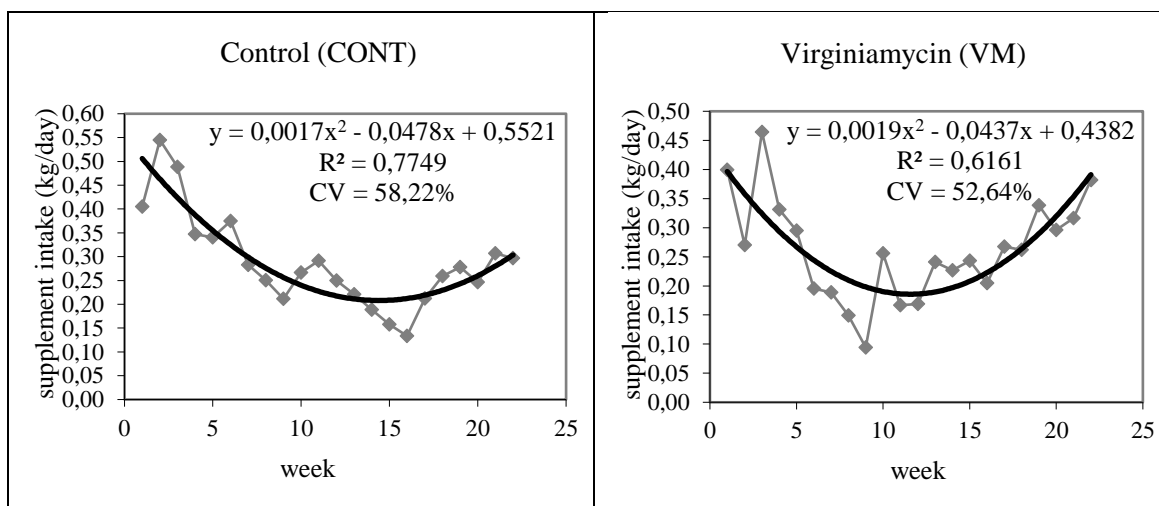
Parameters	Months					Average
	February	March	April	May	June	
Dry Matter	285.6	252.7	210.9	275.5	293.2	263.6
Ash ¹	78.7	86.1	81.6	82.6	84.5	82.7
Organic Matter ¹	921.3	913.9	918.4	917.4	915.5	917.3
Crude Protein ¹	76.4	114.7	158.4	98.2	95.3	108.6
NDIN ¹	23.0	51.7	61.6	32.7	36.2	41.0
NDIN ²	301.2	451.0	388.8	333.3	379.6	370.8
Ether Extract ¹	24.6	24.4	20.6	20.4	24.4	22.9
NDF ¹	630.1	600.4	583.2	597.1	568.3	595.8
ADF ¹	248.5	231.4	225.7	240.3	227.0	234.6
Lignin ¹	48.8	42.8	37.6	46.9	38.6	42.9
Calcium ¹	4.5	4.0	3.7	5.1	6.4	4.7
Phosphorus ¹	1.6	2.1	2.4	1.8	1.5	1.9
TDN ³	575.1	587.5	594.6	588.9	600.8	589.4
NFC ⁴	190.2	174.4	156.2	201.7	227.4	190.0

NDIN: neutral detergent insoluble nitrogen; NDF: neutral detergent fiber; ADF: acid detergent fiber; TDN: total digestible nutrients; NFC: non-fibrous carbohydrate. ¹g/kg DM; ²g/kg CP; ³ TDN=83.39 - 0.4171 NDF (Capelle *et al.*, 2001); ⁴ NFC=100 - (%CP + % NDF + %EE + % Ash) (Sniffen *et al.*, 1992).

Individual supplement intake was not measured in the present study. Nevertheless, a high variability in the daily supplement intake was observed through the experimental period, with coefficient of variation (CV) between 47.75 and 58.22% (Figure 3). The individual supplement intake for cattle on grazing systems presents higher variability for mineral mixtures (CV of 47 to 100%) than for protein or energetic protein supplements (CV of 24 to 37%) (Dixon *et al.*, 2003). Although the supplement used in the present study had considerable amounts of ground corn, the high CV for the supplement intake shows that, in this present study, energy source in mineral supplements did not control the intake variability. We hypothesized that, given the greater daily intake variability an elevated number of animals might have not consumed the supplement daily (Cockwill *et al.*, 2000; Dixon *et al.*, 2003). Thus, strategies to minimize supplement intake variations, avoiding daily variations of additive dose must be evaluated for grazing animals.

There was a reduction of 45.7 and 32.9% in the voluntary intake of supplement due to the inclusion of MON or VM+MON, respectively, in comparison to the CONT group.

This reduction in supplement intake due to the addition of monensin is reported in studies with supplements for grazing cattle (Fieser *et al.*, 2007; Palma *et al.*, 2015), but the mechanisms that result in this inhibition of monensin are not well defined in the literature. On the other hand, VM inclusion resulted in a smaller decrease (9.3%) in supplement consumption, in comparison to the CONT group. The estimated total DMI was 7.96kg/day, equivalent to 2.65% of BW, with no differences between treatments (Table 3), consistent with the predicted DMI calculated using the nutrient requirements by Valadares Filho *et al.* (2010) using the observed BW and ADG in May (0.847kg/animal, day⁻¹). The treatments with additives decreased the supplement intake, however, the total DMI and the ADG during the experimental period were not altered (Table 3). In forage-based diets, monensin usually leads to depression in animals feed intake (Rivera *et al.*, 2010), however, the limited number of studies with grazing animals that presents DMI data makes it difficult to establish relations between monensin and the amount of forage intake (Bretschneider *et al.*, 2008).



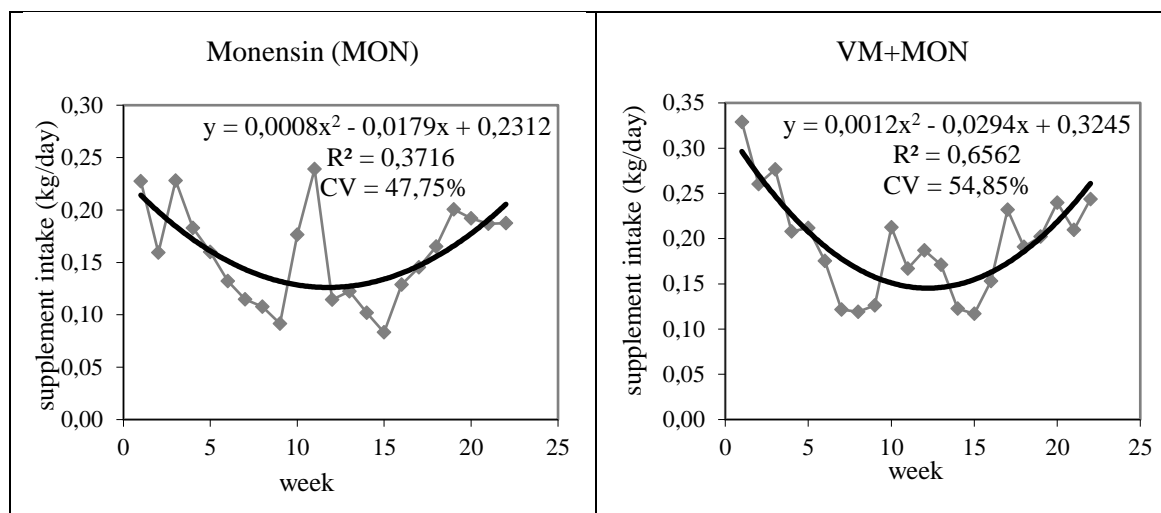


Figure 3. Daily voluntary intake of energetic mineral supplement by the four treatments during the 22 weeks of the rainy season. The regression equations are presented for each treatment. R²: regression coefficient. CV: Coefficient of variation.

Table 3. Dry matter intake (DMI), feed efficiency (FE) and performance of Nellore heifers grazing of *Urochloa brizantha* cv. Marandu receiving energetic mineral supplement added with virginiamycin and/or monensin

Parameters	Treatments ¹				Average	SEM	P-value
	CONT	VM	MON	VM+MON			
ADG ² , kg/day	0.999	0.706	0.761	0.924	0.847	0.06	0.10
DMF ³ , kg/day	8.50	8.22	7.53	7.58	7.96	0.21	0.32
DMI, %BW	2.85	2.78	2.52	2.46	2.65	0,10	0.49
Feed efficiency	0.121	0.087	0.104	0.122	0.108	0.009	0.35
Final weight, kg	336.40	349.17	344.67	333.17	340.85	3.77	0.49
Total ADG, kg/day	0.567	0.600	0.572	0.507	0.561	0.01	0.13

¹CONT= no-additives; VM= virginiamycin at 33.0mg/100kg BW; MON= monensin at 18.8mg/100kg BW; VM+MON= virginiamycin at 26.3 and monensin at 26.3mg/100kg BW. Means within a row with different superscript letters are significantly different by Tukey's test (P ≤ 0.05). ²ADG: average daily gain for the month of provide indicator (may); ³DMI: dry matter intake.

Fieser *et al.* (2007) evaluated supplementation strategies and delivery methods on supplement intake and growth performance of grazing steers and reported that monensin inclusion in the free-choice mineral mixture decreased intake of the mineral mixture by 63% when no other supplement was offered. Petersson-Wolfe *et al.* (2007) evaluated the feed intake variation due to the delivery methods of monensin (controlled-release capsule or in the total mixed ration), and it was found that there were no effects in the DMI between the groups supplemented. Thus, these findings suggest no preference effect in the intake or palatability effects due to the monensin inclusion.

The feed intake reduction has been associated to an increase in a diet net energy which is directly

due to the change in the VFA profile in the rumen-reticulum and the potential increase in amino acids reaching the small intestine (Fox and Black, 1984; Fieser *et al.*, 2007). The supplement intake reduction in VM supplemented group could be explained by the same mechanisms. The nutrients provided by the forage (Table 2), along with the energetic mineral supplementation, allowed an adequate ADG to Nellore raising heifers. The difference in ADG between animals supplemented or non-supplemented with additives was lower when pasture presents increased nutritional value (Bretschneider *et al.*, 2008). Therefore, the forage quality has a great impact in the response of growth promoters use, since the additional ADG lower when the forage quality is better (Duffield *et al.*, 2012).

Total DMI, ADG and feed efficiency (FE) did not differ among treatments (Table 3). The ionophores can increase FE of grazing animals by increasing ADG, due to microbial population changes, to the relative increase of propionate in relation to acetate and the decreased rumen protein deamination (Tedeschi *et al.*, 2011). However, factors such as high variation in the daily intake of antimicrobial, due to the high daily variation of supplement intake and the consequently reduced molecule availability may contribute to the absence of positive results (Cockwill *et al.*, 2000), which might have happened in the present study. Data from the use of monensin for growing and terminating beef cattle were compiled by Duffield *et al.* (2012) in a meta-analysis study. The average monensin dose used in the experiments was 28.1mg per kg of diet and a linear effect was observed for ADG with increasing ionophores dose.

Vendramini *et al.* (2015) evaluated the effects of monensin in crossbreed Angus x Brahman heifers kept in *Paspalum notatum* pasture with a concentrate supplementation restricted to 400mg/animal, day⁻¹ and did not observe differences in ADG between animals supplemented or not with monensin (200mg/animal, day⁻¹), corresponding to 58mg per 100kg BW, a dose above the one consumed by animals in the present study, but with similar results. Vendramini *et al.* (2015) also tested 0, 14.5, 29.0 and 43.5mg of monensin per kg of DMI, using cannulated young bulls, consuming 0.2% of BW of hay and concentrate. These doses corresponded to 0, 31.8, 63.7 and 95.6mg per 100kg BW and no differences were found on DMI. The VM dose is not yet well elucidated in the literature. Alves Neto *et al.* (2013) used 0, 35, 55 and 75mg of VM per 100kg BW in an energetic protein supplement and observed better performance (higher weight and carcass gain) of Nellore grazing consuming 47mg VM per 100kg BW. Goulart (2010) observed that 20.7mg of VM per 100kg BW added to the mineral mixture increased in 16% the weight gain of grazing animals.

Is important to highlight that although the additives did not increase ADG, the lower supplement intake can make their inclusion an economically viable strategy, since it reduces the supplementation cost (R\$/animal, day⁻¹). Considering the supplement costs (Table 1) and

the period of 149 days during the rainy season, the supplementation cost was \$52.10, \$60.50, \$30.17 and \$47.11 per animal for CONT, VM, MON and VM+MON treatments, respectively. Since there was no additive effect on ADG, we can observe that the VM inclusion increased the supplement cost in 28% and since the intake reduction was only 9.3% in relation to the CONT, indicating a higher cost of supplementation. The monensin presented the smaller supplementation cost, once it only increased in 6.6% the supplement cost and reduced supplement intake in 45.7%, without altering the animals' ADG.

CONCLUSIONS

The results did not support the hypothesis that supplementation with antimicrobial additives would increase the performance of grazing Nellore heifers. Supplementation with monensin, virginiamycin, or the association reduces the mineral energy supplement intake with greater reduction caused by monensin. The variability observed in supplement intake and in the daily additive intake by grazing heifers may be influenced in these results. Further research should investigate the factors controlling the intake of mineral supplement and the effects of feeding methods on supplement intake to ensure less variation in the additive intake during the feeding period. Overall, the data from this experiment showed that the inclusion of monensin provides lower costs with supplementation, due to the reduction of intake, without altering the weight gain of the animals.

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