

Associative effect of monensin sodium to virginiamycin on the performance of beef steers in the initial feedlot phase

Efeito associativo da monensina sódica à virginiamicina sobre o desempenho de novilhos de corte na fase inicial de confinamento

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Highlights:

The combined use of monensin sodium and virginiamycin increased daily weight gain.

The apparent digestibility of the diet did not change by supplementation.

Ingestive behavior was not affected by combinations of additives and doses.

Abstract

The objective was to evaluate the associative effect of monensin sodium to virginiamycin on the performance, dry matter intake, apparent digestibility and ingestive behavior of steers in the initial feedlot phase. The experiment lasted for 30 days, divided into two experimental periods. Thirty-six Angus Nellore steers, non-castrated, with a mean age of 10 months and an average weight of 300 kg, were divided into 18 pens. Animals were assigned to three treatments with six replications each, with the inclusion of the following additives: T1-monensin sodium, dose of 200 mg day⁻¹; T2-monensin sodium, dose of 125 mg day⁻¹ + virginiamycin, dose of 125 mg day⁻¹; and T3-monensin sodium, dose of 200 mg day⁻¹ + virginiamycin, dose of 125 mg day⁻¹. All experimental procedures were previously submitted to the UNICENTRO Committee for Ethics in Animal Experimentation (CEUA), and were approved for execution (Official Letter 021/2019). The combination of monensin sodium with virginiamycin at a dose of 200 mg + 125 mg animal day⁻¹ showed greater ($P < 0.05$) average daily weight gain (1.919 kg day⁻¹) and better feed conversion (4.27 kg DM kg of weight gain⁻¹) compared to diets with monensin alone (200 mg animal day⁻¹) or monensin combined with virginiamycin (125 mg + 125 mg animal day⁻¹), even with no significant ($P > 0.05$) difference in DM intake, with a mean value of 7.88 kg animal day⁻¹. Regardless of the isolated or combined supplementation of the additives, no significant differences were detected in the analysis of the feeder and feces scores, ingestive behavior, and rectal temperature or infrared thermography during the evaluation period. The combination of monensin sodium with virginiamycin at 200 mg + 125 mg animal day⁻¹ proved to be efficient in the initial feedlot period,

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determined by the greater weight gain and better feed conversion observed in this study.

Key words: Bovine. Feedlot adaptation phase. Food additives. Performance enhancers.

Resumo

O objetivo foi avaliar o efeito associativo da monensina sódica à virginiamicina sobre o desempenho, consumo de matéria seca, digestibilidade aparente e comportamento ingestivo de novilhos na fase de inicial de confinamento. A duração do experimento foi de 30 dias, divididos em dois períodos experimentais de 15 dias cada. Utilizou-se de 36 novilhos inteiros ½ sangue Angus Nelore, com idade média 10 meses e peso médio de 300 kg, divididos em 18 baias. Os animais foram divididos em três tratamentos com seis repetições cada, com a associação dos seguintes aditivos incluídos as rações: T₁ – monensina sódica, dose de 200 mg dia⁻¹; T₂ – monensina sódica, dose de 125 mg dia⁻¹ + virginiamicina, dose de 125 mg dia⁻¹; e T₃ – monensina sódica, dose de 200 mg dia⁻¹ + virginiamicina, dose de 125 mg dia⁻¹. Todos os procedimentos experimentais foram previamente submetidos à apreciação do Comitê de Conduta Ética no Uso de Animais em Experimentação (CEUA) da UNICENTRO, tendo sido aprovados para execução (Ofício nº 021/2019). A associação de monensina sódica com virginiamicina na dose de 200 mg + 125 mg animal dia⁻¹ apresentou maior (P < 0,05) ganho de peso médio diário (1,919 kg dia⁻¹) e melhor conversão alimentar (4,27 kg de MS kg de ganho de peso⁻¹) comparativamente as dietas com monensina isolada (200 mg animal dia⁻¹) ou monensina associada a virginiamicina (125 mg + 125 mg animal dia⁻¹), mesmo não tendo sido observada (P > 0,05) diferença na ingestão de MS, apresentando valor médio de 7,88 kg animal dia⁻¹. Independentemente da suplementação isolada ou associada dos aditivos, não encontrou-se estatisticamente diferenças na análise dos escores de comedouro e fezes, comportamento ingestivo e temperatura retal ou termografia infravermelha durante o período avaliativo. A associação de monensina sódica com virginiamicina na dose de 200 mg + 125 mg animal dia⁻¹ demonstrou ser eficiente no período inicial do confinamento, determinado pelo maior ganho de peso e melhor conversão alimentar verificados neste estudo.

Palavras-chave: Aditivos alimentares. Bovino. Fase de adaptação ao confinamento. Melhoradores de desempenho.

Introduction

Cattle ranching in Brazil has undergone intense changes with regard to the development of strategies that allow the intensification of the meat production chain. The use of confinement and supplementation with food additives are widespread techniques, however some factors, such as the range of possible additive combinations, open up an array of possibilities for their use at different times during the finishing period.

According to Marques, Cooke, Francisco and Bohnert (2012), the adaptation period of cattle deserves special attention from nutritionists, as these animals are currently challenged by the stress generated mainly by transportation, thus requiring nutritional practices aimed at favoring the animal performance and health. This period represents

about 20% of the total feedlot time (Brown & Millen, 2009).

Among the additives most used in animal nutrition, are ionophores, which come from several strains of *Streptomyces*, and monensin sodium (MO) is the most common (Soares, Silva, Frazão, & Silva, 2015).

The selectivity generated by the use of MO decreases the population of gram-positive bacteria, allowing an increase in ruminal propionate synthesis, resulting in a decrease in the proportion of acetate, without significantly affecting energy production (Beacom & Mir, 1985). Another action of MO is related to the possibility of reducing ruminal protein degradation, varying according to the quality of the protein supplied, thus resulting in less loss through the synthesis of ammonia, consequently greater

amount of protein of high biological value would reach the small intestine for absorption (Zanine, Oliveira, & Santos, 2006).

This change in the rumen microbiome determines the improvement in cattle performance, as there are changes in the proportion of short chain fatty acids (SCFA), with an increase in the molar proportion of propionate and a reduction in butyrate and acetate (Vendramini et al., 2016). Other effects caused by the use of ionophores are increased energy retention, reduced dry matter intake, and increased daily weight gain and feed efficiency (Duffield, Merrill, & Bagg, 2012).

Oliveira and Millen (2014) report that 99.2% Brazilian feedlots use additives in their diets, and that approximately 93.9% use ionophore antibiotics. Therefore, it is necessary to define the combinations that may be convenient. Thus, a possibility of use that can act in synergy with ionophores is virginiamycin (VM).

VM is a non-ionophore antibiotic of the streptogramin class produced by a mutant strain of *Streptomyces virginiae* (De Somer & Van Duck, 1955). Its activity mainly affects gram-positive bacteria, both aerobic and anaerobic, however, it has no effect on most gram-negative bacteria due to the impermeability of the cell membrane (Cocito, 1979).

VM is composed of two chemical components: factor M (C₂₅H₃₅N₃O₇) and factor S (C₄₃H₄₉N₇O₁₀) (Crooy & De Neys, 1972), with antibacterial activity dependent on the synergy between its two components, as long as their combination follows the 4: 1 ratio (Vanderhaeghe & Parmentier, 1960). After the entry of VM into gram-positive cells, it irreversibly binds to a ribosomal subunit (50S subunit), inhibiting the formation of the peptide bond during bacterial protein synthesis (DiGiambattista, Chinali, & Cocito, 1989), leading to disruption of cellular metabolic processes and consequent bacteriostasis and death.

As a background to the levels of supplementation of the additives tested here, the Food and Agriculture Organization of the United Nations (FAO, 2009) determines that the maximum recommended dose of MO aimed at efficiency in ruminal fermentation is 360 mg day⁻¹ or for confined animals of 40 mg kg⁻¹ in the ready feed, whereas for VM, the maximum dose recommended by the Ministry of Agriculture, Livestock and Food Supply (MAPA) is 75 mg VM 100 kg⁻¹.

The action of antimicrobials in the ruminant diet has led to several studies seeking to measure their effects on animal production, however little research has been conducted to assess the associative effect of ionophore and non-ionophore antibiotics, their different dosages and effects on animal performance in the adaptive period. Therefore, the goal of the study was to evaluate the associative effect of monensin sodium combined with virginiamycin on performance, dry matter intake, apparent digestibility and ingestive behavior of steers in the initial feedlot phase.

Material and Methods

The experiment was carried out at the Animal Production Nucleus (NUPRAN, Núcleo de Produção Animal), in the Graduate Program in Veterinary Sciences, area of Sustainable Animal Production and Health, Agricultural and Environmental Sciences Sector, State University of the Midwest (UNICENTRO), in the city of Guarapuava, State of Paraná. All experimental procedures were previously submitted to the UNICENTRO Committee for Ethics in Animal Experimentation (CEUA), and approved for execution (Official Letter 021/2019).

The dry matter intake, animal performance, apparent digestibility and ingestive behavior were evaluated in steers undergoing adaptation to feedlot and receiving the following additives included to feed: Treatment 1 - monensin sodium, 200 mg day⁻¹; Treatment 2 - monensin sodium, 125 mg day⁻¹

+ virginiamycin, 125 mg day⁻¹; and Treatment 3 - monensin sodium, 200 mg day⁻¹ + virginiamycin, 125 mg day⁻¹.

Thirty-six steers, ½ Angus Nellore blood, non-castrated males, from the same herd, with initial average weight of 300 kg and average age of 10 months were housed in 18 feedlot pens, 2 animals per pen, semi-roofed, with 15 m² area, with a concrete feeder and automatic metal water trough, regulated by a float.

The experiment lasted for 30 days, divided into two 15-day evaluation periods, which refers to the adaptation of animals to feedlot, diets and facilities.

Feed was supplied twice a day, at 06 h: 00 a.m. and 05 h:30 p.m. as a complete mixed ration (RTM). The diets consisted of corn silage at a constant ratio of 40% roughage and 60% concentrate, on a dry matter basis. The adjustment in the supply was carried out daily, considering leftovers of 5%, on a diet dry matter basis. The additives were supplied on the complete mixed ration, previously weighed and mixed with 50 g ground concentrate, in order to guarantee their total ingestion.

The concentrate was prepared in the commercial feed factory of Cooperativa Agrária (Guarapuava, State of Paraná, Brazil), formulated based on soybean meal, corn, wheat meal, soybean husk, malt root, calcitic limestone, dicalcium phosphate, livestock urea, common salt, and mineral vitamin premix, presented in pellets. The guarantee levels of premix per kg concentrate are as follows: vitamin A: 16,000 IU; vitamin D3: 2,000 IU; vitamin E: 25 IU; S: 0.36 g; Mg: 0.74 g; Na: 3.6 g; Co: 0.52 mg; Cu: 22.01 mg; Fe: 18.00 mg; I: 1.07 mg; Mn: 72.80 mg; Se: 0.64 mg; and Zn: 95.20 mg.

During the 30 days of adaptation to the feedlot, samples of corn silage and concentrate were taken to determine the chemical composition of the diet (Table 1). Samples were dried in a ventilated oven at 55°C to constant weight and sequentially ground in

a Wiley mill with a 1 mm diameter sieve. Analysis of dry matter (DM), crude protein (PB), mineral matter (MM) and ether extract (EE) were performed according to Association of Official Analytical Chemists [AOAC] (1990). The content of neutral detergent fiber (NDF) was obtained according to the Van Soest method (1991) with thermostable α -amylase, and acid detergent fiber (ADF) and lignin (LIG), according to Goering and Van Soest (1970). For the determination of the contents of P and Ca, analyses were performed according to Tedesco, Gianello, Bissani, Bohnen and Volhweiss (1995). The coefficient of total digestible nutrients (TDN) was calculated according to Weiss, Conrad and Pierre (1992) and the concentration of non-fiber carbohydrates (NFC) was calculated by difference, according to the formula: $NFC = 100 - (MM + CP + EE + NDF)$, as indicated by AOAC (1990).

The product used based on monensin sodium (MO) was Rumensin[®] 200, produced by the company Elanco Saúde Animal, registered at MAPA under number SP-59410 30002, which is classified as an animal performance enhancer, and the product based on virginiamycin (VM) used was Eskalin[®] (20 g kg⁻¹ virginiamycin), registered at MAPA by the company Phibro Saúde Animal Internacional Ltda. under number SP-09492 30012, which is classified as a performance-enhancing additive composed of virginiamycin and 99% calcium carbonate.

Animals were weighed at the beginning, middle and at the end of the experiment, after solid fasting for 10 hours, to determine the average daily weight gain (ADG). The daily dry matter intake, expressed in kg day⁻¹ (DDMI) or expressed as a percentage of body weight (DMIP) was measured by means of the difference between the daily amount of food supplied and the amount of leftover food from the previous day. To determine feed conversion (FC), we calculated the ratio of the average daily dry matter intake and the average daily weight gain obtained in the evaluation period (DDMI ADG⁻¹).

Table 1
Chemical composition of ingredients used in animal feed and average values of the experimental diet, on a total dry matter basis

Parameter	Corn silage	Concentrate	Experimental diet
Dry matter, %	41.37	90.57	70.89
Mineral matter, % DM	2.99	5.40	4.44
Ether extract, % DM	2.96	3.45	3.25
Crude protein, % DM	6.26	18.32	13.50
Neutral detergent fiber, % DM	53.18	25.18	36.37
Acid detergent fiber, % DM	28.42	10.03	17.39
Lignin, % DM	6.73	2.45	4.16
Non-fiber carbohydrates, % DM	34.61	47.66	42.44
Total digestible nutrients, %	67.94	80.82	75.67
Ca, %	0.14	1.67	0.91
P, %	0.22	0.58	0.40

DM: dry matter, Ca: calcium, P: phosphorus.

In the middle of the adaptation period of the steers to the feedlot, the total collection of feces from each experimental unit was performed for 48 consecutive hours, concomitantly with the behavioral assessment. The feces collected at the end of each 6-hour shift were weighed individually per experimental unit and 500 g were taken to be dried in a ventilated oven at 55°C to constant weight. This procedure allowed to determine the fecal dry matter content (FDM), and sequentially to estimate the total fecal output on a dry matter basis (FTS), expressed in kg day⁻¹ dry matter.

In order to obtain the apparent digestibility of dry matter (DMD), homogeneous samples of the diet and feces were taken for further analysis. The DMD, expressed in g kg⁻¹ DM, was calculated using the formula: Digestibility $\{1 - [(nutrient\ ingested - nutrient\ excreted) \div nutrient\ ingested]\} \times 100$.

Ingestive behavior was analyzed in the middle of the experiment, during 48 consecutive hours of observation, starting at 12 noon on the first day and ending at 12 noon on the third day. The visualizations were made by four observers per shift, in a rotation

system every 6 hours. The readings were taken at regular intervals of 3 minutes, in order to maintain the accuracy in the monitoring regarding the time spent in each activity. Ingestive behavior was represented by idle, rumination, water intake and food intake activities, expressed in hours day⁻¹.

The observations were recorded in two different worksheets, one for assessing the time spent in each activity, and the other for recording the number of times that each activity was performed. This methodology allows the determination of the frequency of activities (feeding, drinking, urination and defecation), expressed in number of times per day. In the night observation, the environment was maintained with artificial lighting, and the animals were previously adapted for this.

During the 30 days of the experiment, feces of each pen were evaluated daily by means of visual observation scores. Feces were classified by scores, ranging from 1 to 6, in which: 1 = liquid feces, mushy; 2 = liquid feces, mushy, with small piles of up to 2.5 cm; 3 = intermediate feces with concentric ring and 3 to 4 cm pile; 4 = pasty feces

with concentric ring and pile of more than 5 cm; 5 = drier stools without concentric ring and pile greater than 5 cm; 6 = hardened or dried stools, based on the methodology adapted from Looper, Stokes, Waldner and Jordan (2001) and Ferreira et al. (2013).

In addition to the ranking of feces scores, we also visually evaluated daily the dietary leftovers in relation to the proportion of corn silage and concentrate, on a dry matter basis. The leftovers were ranked by scores, ranging from 1 to 6, where 1 = 60% silage and 40% concentrate; 2 = 50% silage and 50% concentrate; 3 = 40% silage and 60% concentrate; 4 = 30% silage and 70% concentrate; and 5 = 20% silage and 80% concentrate; 6 = 10% silage and 90% concentrate, on a dry matter basis.

Aiming to evaluate the influence of treatments on possible inflammatory conditions or clinical signs of diseases, the temperature of the left front limb (hull region) and the central superficial skin region of the rumen was measured twice a week, on pre-set days and times (02h00 p.m.) with FLUKE infrared camera, Ti100. At the end of each experimental period, at the time of weighing, the animals' rectal temperature was also measured with a Bioland digital thermometer.

Data obtained for each variable were tested for normality by the "Shapiro-Wilk" test (PROC UNIVARIATE) and for homogeneity by the "Bartlett" test (PROC GLM). Subsequently, the results obtained were tested by analysis of variance, and subsequently means were compared by Tukey's test at 5% significance, through the GLM procedure of SAS (Statistical Analysis System Institute [SAS

Institute], 1993). The experiment was a 3 x 2 factorial randomized block design, with three combinations of additives and two evaluation periods, with six repetitions, where each repetition (block) was represented by a pen with two animals each. The following statistical model was used: $Y_{ijk} = \mu + S_i + P_j + B_k + (S*P)_{ij} + E_{ijk}$; Where: Y_{ijk} = dependent variable; μ = overall mean of all observations; S_i = Effect of additive "i", where 1 = control diet with monensin sodium, dose of 200 mg day⁻¹, 2 = diet with monensin sodium, dose of 125 mg day⁻¹ + virginiamycin, dose of 125 mg day⁻¹, and 3 = diet with monensin sodium, dose of 200 mg day⁻¹ + virginiamycin, dose of 125 mg day⁻¹; P_j = Effect of the feedlot period of order "j", with 1 = first period and 2 = second period; B_k = Effect of the block of order "k", where 1 = first, 2 = second, 3 = third, 4 = fourth, 5 = fifth and 6 = sixth; $(S*P)_{ij}$ = Effect of the interaction between additive dose and feedlot period of order "ij" and E_{ij} = Residual random effect.

Results and Discussion

Based on the summary of the analysis of variance (Table 2), there was no interaction ($P > 0.05$) between the combination of additives and the feedlot period assessed, for the following parameters: Average daily weight gain (ADG), dry matter intake (DMI), expressed in kg day⁻¹ (DDMI) or per 100 kg body weight (DMIP), feed conversion (CA), feeder score (FdS) and feces score (FS) and for temperatures of the left front limb (TMAE), central superficial skin region of the rumen (TCR) and of the rectum (TR).

Table 2
Summary of analysis of variance for average daily weight gain (ADG), dry matter intake (DMI), expressed in kg day⁻¹ (DDMI) or per 100 kg body weight (IMSP), feed conversion (FC), feeder score (FdS), and feces score (FS), and for temperatures of the left front limb (TMAE), central superficial skin region of the rumen (TCR) and of the rectum (TR), of steers in the initial feedlot phase supplemented with monensin sodium in combination with virginiamycin included in the diet, according to the feedlot period

Source of variation	Mean square of the error					Probability (P<0.05)						
	Additive (A)	Period (P)	Block (B)	A*P	Error	R ²	CV, %	Mean	A	P	B	S*P
DF*	2	1	5	2	25	-	-	-	-	-	-	-
Performance measurements:												
ADG	0.2807	1.3353	0.0325	0.2526	0.1238	0.4532	20.16	1.744	0.0244	0.0030	0.9290	0.1510
DDMI	0.4490	6.2167	0.3342	0.0469	0.4827	0.4238	8.81	7.88	0.4077	0.0014	0.6340	0.9076
IMSP	0.0153	0.0187	0.0223	0.0040	0.0299	0.1844	7.44	2.32	0.6050	0.4368	0.5961	0.8749
FC	2.1378	6.0762	0.6314	2.8498	1.2120	0.3880	23.14	4.76	0.0421	0.0343	0.7581	0.1160
FdS	0.0408	0.5136	0.1598	0.0086	0.0606	0.4823	11.23	2.19	0.5190	0.0075	0.0478	0.8683
FS	0.0033	0.0544	0.0533	0.0044	0.0149	0.4742	4.05	3.02	0.8015	0.0678	0.0142	0.7452
Temperatures												
TMAE	1.9519	10.1336	8.1003	2.0836	3.9647	0.4037	6.87	27.11	0.5767	0.0998	0.0718	0.5562
TCR	1.4711	4.8400	13.2038	0.0033	0.6587	0.8175	2.54	31.91	0.1281	0.1120	0.0001	0.9950
TR	0.9769	1.8225	1.4723	0.5416	0.4636	0.5021	1.94	38.47	0.1425	0.0684	0.0235	0.5648

* DF: degrees of freedom.

The combination MO in the dose of 200 mg + VM 125 mg animal day⁻¹ provided greater ($P < 0.05$) average daily weight gain (1.919 kg day⁻¹) and better feed conversion (4.27 kg DM kg weight gain⁻¹) compared to diets with MO alone at the dose of 200 mg animal day⁻¹ (1.636 kg day⁻¹ and 5.06 kg DM kg weight gain⁻¹) or MO at the dose of 125 mg animal day⁻¹ combined with VM at a dose of 125 mg animal day⁻¹ (1.678 kg day⁻¹ and 4.94 kg DM kg weight gain⁻¹). Regarding the mean values of dry matter intake, these remained constant ($P > 0.05$) in the different treatments, regardless of the supplementation of additives (Table 3).

In the general mean, there was a positive highlight in the first 15 days of feedlot for the experimental group that received MO combined with VM (200 mg + 125 mg animal day⁻¹) for determining greater ($P < 0.05$) average daily weight gain and better feed conversion compared to diets with MO alone (200 mg animal day⁻¹) or OM combined with VM (125 mg + 125 mg animal day⁻¹). In turn, 15 days after the beginning of the feedlot, the parameters related to the animal performance and dry matter intake were

similar ($P > 0.05$) between the different treatments, no longer observing advantages of the combination of MO with VM in the dose of 200 mg + 125 mg animal day⁻¹.

Guan, Wittenberg, Ominski and Krause (2006) found that alternating supplementation of MO and lasalocide, and also the use of MO alone did not alter the ADG (0.90 and 0.89 kg day⁻¹) and the DMI of one-year-old steers receiving a low concentrate diet. The same authors investigated supplementation with ionophores in a diet with a proportion of 31% roughage and 69% concentrate and found better results in FC for both additives, with 7.23 kg DM kg weight gain⁻¹ for the group receiving MO and 7.11 kg DM kg weight gain⁻¹ for the group receiving the combination of MO and lasalocide. These data corroborate the present study regarding the improvement of FC when MO was combined with another additive, with values of 4.27 kg DM kg weight gain⁻¹ found for the combination of MO with VM (200 mg + 125 mg animal day⁻¹) and 5.06 kg DM kg weight gain⁻¹ for MO alone (200 mg animal day⁻¹) in the present study.

Table 3
Average daily weight gain, daily dry matter intake and feed conversion of steers in the initial feedlot phase supplemented with monensin sodium combined with virginiamycin included in the diet, according to the feedlot period

Experimental diet	Phase of adaptation to the feedlot		Mean
	1st period 1-15 days	2nd period 16-30 days	
	Average daily weight gain, kg animal day ⁻¹		
MO, 200 mg day ⁻¹	1.361	1.911	1.636 B
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	1.400	1.956	1.678 B
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	1.894	1.944	1.919 A
Mean	1.552 b	1.937 a	
	Dry matter intake, kg animal day ⁻¹		
MO, 200 mg day ⁻¹	7.49	8.40	7.95 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	7.32	8.01	7.66 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	7.58	8.48	8.03 A
Mean	7.46 b	8.30 a	

continue

continuation

	Dry matter intake per 100 kg body weight, %		
MO, 200 mg day ⁻¹	2.32	2.39	2.35 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	2.28	2.29	2.29 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	2.31	2.37	2.34 A
Mean	2.30 a	2.35 a	
	Feed conversion (Dry matter intake Weight gain ⁻¹)		
MO, 200 mg day ⁻¹	5.60	4.51	5.06 B
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	5.75	4.12	4.94 B
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	4.14	4.40	4.27 A
Mean	5.17 b	4.35 a	

Mean values followed by different lowercase letters, in the same row, are significantly different by F-Test at 5%.

Mean values followed by different capital letters, in the same column, are significantly different by Tukey's test at 5%.

MO: monensin sodium, VM: virginiamycin.

Although MO is the most researched ionophore, salinomycin in studies that evaluate performance parameters has generated results close to MO (Gibb, Moustafa, Wiedmeier, & McAllister, 2001). In an experiment using Nellore steers, supplemented with 13 mg kg⁻¹ salinomycin combined or not with VM (15 mg kg⁻¹) fed a diet composed of 73% concentrate, Nuñez et al. (2013) reported a decline in the DMI of the animals that received the combination of additives (6.85 kg day⁻¹), differently from what was observed in the present study. The ADG found by the same author showed no synergy between the additives, remaining at 1.240 kg day⁻¹.

Heker et al. (2018) examined the performance of confined steers receiving MO combined with VM and/or essential oils and found that the ADG for treatment with MO was 1,329 kg day⁻¹ versus 1,559 kg day⁻¹ in the combination of MO and VM, these data are in line with data of the present experiment with 1,636 kg day⁻¹ versus 1,799 kg day⁻¹ of the average of the treatments receiving different doses of MO and VM, that is, the combination of these provided greater daily gain regardless of the dosage provided.

According to Bergen and Bates (1984), ionophores are capable of reducing the synthesis of lactic acid, thus avoiding metabolic disorders like

acidosis. The remarkable role of VM in modifying the microbial population favors the stabilization of rumen pH (Nuñez et al., 2013). Thus, it is inferred that the improvement in ruminal health, especially important in diets containing a higher concentrate content, favored the increase in ADG in animals that received the combination of MO and VM (200 mg + 125 mg animal day⁻¹).

Besides that, according to Sitta (2011), VM is responsible for reducing the synthesis of methane, decreasing energy losses, a fact that adds up to the better performance of animals whose treatments had VM.

Assessing the supply of VM and salinomycin to grazing animals, Ferreira (2013) found an increase of 118 grams animal⁻¹ in the ADG for the group receiving VM, in relation to the control treatment (0.583 kg day⁻¹ versus 0.465 kg day⁻¹), and 52 grams animal day⁻¹ in supplementation with salinomycin (0.583 kg day⁻¹ versus 0.531 kg day⁻¹).

Silva, Almeida, Schwahofer, Leme and Lanna (2004) also did not observe an improvement in the ADG of animals receiving VM or salinomycin alone, but when supplied in combination, there was an increase in ADG, as observed in this study in the group that received the combination of MO with VM (200 mg + 125 mg animal day⁻¹).

Regarding the mean values of dry matter intake (DMI), these remained constant ($P > 0.05$) in the different treatments evaluated, regardless of additive supplementation.

Although Tedeschi, Fox and Tylutki (2003) cite that it is common the lower intake by animals that received MO in the diet, the moderate level of inclusion tested in the present study may have been responsible for not observing this reduction in DMI, despite having provided increased ADG in combination with VM (200 mg + 125 mg animal day⁻¹).

Pereira et al. (2015) found a decrease in the mean values of DMI in animals receiving MO in different dosages compared to the control group (10.66 kg animal day⁻¹ against 11.23 kg animal day⁻¹). These results differ from the present study, where there was no difference ($P > 0.05$) for DMI between treatments, including the combinations of MO with VM. The same result was reported by Rogers et al. (1995), where there was an increase in animal performance without affecting the dry matter intake.

Heker et al. (2018), when evaluating the DMI of steers confined and supplemented with MO alone

or MO combined with VM, also did not obtain any difference between treatments. Squizatti (2019) evaluated 5 cannulated cattle, adapted for 14 days and supplemented with MO alone (30 mg kg⁻¹) or MO + VM (30 mg kg⁻¹ + 25 mg kg⁻¹) and found no differences in DMI corroborating the present experiment.

In the general mean, no effects ($P > 0.05$) from the use of MO were detected, alone or when combined with VM in relation to leftover in feeders and feces scores in the initial phase of confined steers (Table 4).

In the evaluation between feedlot periods, regardless of the use of additives, a lower feeder score ($P < 0.05$) was found in the initial period, compared to the second period (2.1 versus 2.3), that is, the animals tended to ingest a little more of the bulky fraction of the diet initially, since the supply followed the 60:40 ratio, but without differences ($P > 0.05$) between the different treatments. As for the feces scores, these remained stable ($P > 0.05$) in the two evaluation periods and during the adaptation of steers to the feedlot.

Table 4

Average fecal output, on the natural and dry matter basis in kg day⁻¹, apparent digestibility of feed and ingestive behavior regarding aspects of feeder score and feces score of steers in the initial feedlot phase supplemented with monensin sodium combined with virginiamycin included in the diet

Experimental diet	Fecal output kg MN day ⁻¹	Fecal output kg DM day ⁻¹	DM digestibility %
MO, 200 mg day ⁻¹	13.75 A	2.56 A	66.52 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	13.49 A	2.51 A	66.69 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	14.94 A	2.80 A	65.77 A
Mean	14.06	2.62	66.33
Probability	0.4242	0.1171	0.1522
CV, %	13.84	4.25	3.24
Phase of adaptation to the feedlot			
Experimental diet	1st period 1-15 days	2nd period 16-30 days	Mean

continue

continuation

	Daily feeder score		
MO, 200 mg day ⁻¹	2.1	2.3	2.2 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	2.0	2.2	2.1 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	2.1	2.3	2.2 A
Mean	2.1 b	2.3 a	
	Daily feces score		
MO, 200 mg day ⁻¹	3.0	2.9	3.0 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	3.0	3.0	3.0 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	3.1	3.0	3.0 A
Mean	3.0 a	3.0 a	

Mean values followed by different capital letters, in the same column, are significantly different by Tukey's test at 5%. MO: monensin sodium, VM: virginiamycin, MN: natural matter, DM: dry matter.

There was no variation ($P>0.05$) for the parameters related to the average fecal output, on the natural and dry matter basis in kg day⁻¹ and apparent digestibility of the feed, with mean values of 14.06 kg MN day⁻¹, 2.62 kg DM day⁻¹ and 66.33%, respectively. These data corroborate Fonseca (2012), who found no statistical difference for apparent digestibility of the diet in a 50:50 proportion, in steers supplemented with a combination of MO and VM.

Zeoula et al. (2008) analyzed the supplementation with MO in diets with 50% concentrate in the diet, and obtained a value of 61% for in vitro digestibility of DM. In an experiment carried out by Fereli et al. (2010), in a diet with 70% concentrate and 30% roughage, and supplementation with 200 mg day⁻¹ MO, obtained 64.17% apparent digestibility of the

diet, these results suggest a better action of MO in diets with higher content of concentrate

There were no ($P> 0.05$) effects of using MO alone or in combination with VM regarding ingestive behavior (expressed in hours day⁻¹ or number of times day⁻¹) (Table 5).

As for the rumination behavior and time, an experiment carried out by Sitta (2011) showed no difference with the inclusion of MO and/or MO combined with VM. According to Heker et al. (2018), these results can be explained by the same physical characteristic of the feed, being different only the supplementation offered to the animals. Pereira et al. (2015) evaluated the diet with inclusion of MO in different dosages, and also observed no difference in the number of trips to the feeder, with an average value of 15.53 times day⁻¹.

Table 5
Ingestive behavior (hours day⁻¹ and number of times day⁻¹) of steers in the initial feedlot phase supplemented with monensin sodium combined with virginiamycin included in the diet

Experimental diet	Ingestive behavior			
	Feeding	Drinking	Ruminating	Idle
	Hours day ⁻¹			
MO, 200 mg day ⁻¹	2.74 A	0.17 A	5.69 A	15.45 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	3.05 A	0.24 A	5.22 A	15.52 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	2.98 A	0.24 A	5.53 A	15.28 A
Mean	2.93	0.22	5.48	15.42
Probability	0.4360	0.5405	0.4679	0.8355
CV, %	14.62	127.00	11.64	4.62

	Ingestive behavior			
	Feeding	Drinking	Defecating	Urinating
	Number of times day ⁻¹			
MO, 200 mg day ⁻¹	15.50 A	6.58 A	6.33 A	4.92 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	15.25 A	5.58 A	6.25 A	4.25 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	15.83 A	4.50 A	7.50 A	5.50 A
Mean	15.53	5.56	6.69	4.89
Probability	0.9395	0.4061	0.5598	0.2171
CV, %	18.41	26.23	22.60	23.44

Mean values followed by different capital letters, in the same column, are significantly different by Tukey's test at 5%.
 MO: monensin sodium, VM: virginiamycin.

In the present experiment, there was no ($P > 0.05$) effect of the use of MO alone or in combination with VM regarding variations in the temperature of the left front limb, rumen and of the rectum of steers

during the phase of adaptation to feedlot, as well, temperature values did not change ($P > 0.05$) during the evaluation period (Table 6).

Table 6
Temperature of the left front limb, central superficial skin region of the rumen and rectum of steers in the initial feedlot phase supplemented with monensin sodium in combination with virginiamycin included in the diet, according to the feedlot period

Experimental diet	Phase of adaptation to the feedlot		Mean
	1st period 1-15 days	2nd period 16-30 days	
	Left front limb, °C		
MO, 200 mg day ⁻¹	28.21	26.71	27.46 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	26.70	26.63	26.67 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	28.01	26.44	27.23 A
Mean	27.64 a	26.59 a	
	Rumen, °C		
MO, 200 mg day ⁻¹	32.28	31.49	31.88 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	32.63	31.92	32.27 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	31.91	31.21	31.56 A
Mean	32.27 a	31.54 a	
	Rectum, °C		
MO, 200 mg day ⁻¹	38.44	38.43	38.43 A
MO, 125 mg day ⁻¹ + VM, 125 mg day ⁻¹	38.14	38.50	38.32 A
MO, 200 mg day ⁻¹ + VM, 125 mg day ⁻¹	38.71	38.66	38.68 A
Mean	38.43 a	38.53 a	

Mean values followed by different lowercase letters, in the same row, are significantly different by F-Test at 5%.

Mean values followed by different capital letters, in the same column, are significantly different by Tukey's test at 5%.

MO: monensin sodium, VM: virginiamycin.

The metabolic rate of the tissues and the blood flow determine the surface temperature of the animals (Nikkhah et al., 2005), therefore several diseases related to inflammatory processes can consequently alter the blood flow and thus, the temperature of the affected region (Berry, Kennedy, Scott, Kyle, & Schaefer, 2003). According to Bouzida, Bendada and Maldague (2009), these changes in the skin surface can be verified using infrared thermography (TIV).

The use of TIV has been growing, due to the non-invasiveness of the technique, and it can be used without restraining the animals (Schaefer et al., 2012). Further, it can serve to prevent diseases, by identifying the rise in temperature even before

the appearance of clinical signs, allowing thorough observation of the animal (Gloster, Ebert, Gubbins, Bashiruddin, & Paton, 2011); Redaelli et al., 2014).

Based on the above, it is assumed that MO alone or combined with VM added to the diet for ruminants did not cause vascular changes, capable of altering body surface temperature.

Conclusions

The combination of monensin sodium with virginiamycin at a dose of 200 mg + 125 mg animal day⁻¹ is efficient in the initial period of finishing steers in feedlot, determined by daily weight gain and feed conversion.

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