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USE OF VIRGINIAMYCIN AND SALINOMYCIN IN THE DIET OF BEEF CATTLE REARED UNDER GRAZING DURING THE RAINY SEASON: PERFORMANCE AND RUMINAL METABOLISM

USO DE VIRGINIAMICINA E DE SALINOMICINA NA DIETA DE BOVINOS DE CORTE CRIADOS EM SISTEMA DE PASTEJO NO PERÍODO DAS ÁGUAS: DESEMPENHO E METABOLISMO RUMINAL

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Abstract

The rumen is the most studied organ with regard to the symbiotic interaction between host and microbiota in the digestive tract of ruminants. Thus, this study aimed at evaluating the effects of including virginiamycin and salinomycin to the supplement for cattle fed tropical grass diet during the rainy season. Three treatments were set: control – mineral supplement COMIGO - Cria 61-F2 (MS); virginiamycin – MS + virginiamycin (Phigrow ®) 100 mg/animal/day; salinomycin – MS + salinomycin (Posistac®) 108 mg/animal/day for the experiment 1 (performance) and experiment 2 (ruminal metabolism). The control showed higher Mineral supplement intake (MSI) ($P < 0.05$), the virginiamycin had higher average daily gain (ADG) ($P > 0.05$) and better financial efficiency. Biometric measurements showed no differences ($P > 0.05$), suggesting a change in the ADG composition. There was no difference ($P > 0.05$) for dry matter digestibility (DMDIS), acid detergent fiber digestibility (ADFDIS), ruminal pH and ammoniacal-N. The virginiamycin had the highest effective neutral detergent fiber (NDF) degradability ($P > 0.05$) in the passage rates of 2 and 5%; the degradability rates were similar to control treatement and higher than that of salinomycin at the rate of 8%/hour. The virginiamycin or salinomycin can be conveyed to the MS, but do not promote significant effects on ruminal pH, ammoniacal-N, DMDIS and ADFDIS; but virginiamycin promoted greater effective degradability of NDF.

Keywords: ammoniacal-N; antimicrobials; degradability; growth; ruminal pH.

Resumo

O rúmen é o órgão mais estudado no que se refere à interação simbiótica entre hospedeiro e a

microbiota do trato digestivo dos ruminantes. Assim, objetivou-se avaliar os efeitos da inclusão de virginiamicina e de salinomicina ao suplemento para bovinos de corte com dieta de gramínea tropical no período das águas. Foram constituídos três tratamentos: controle - suplemento mineral COMIGO – Cria 61 – F2 (SM); virginiamicina - SM + virginiamicina (Phigrow®) 100 mg/animal/dia; salinomicina - SM + salinomicina (Posistac®) 108 mg/animal/dia, para o experimento 1 (desempenho) e experimento 2 (metabolismo ruminal). O controle apresentou maior CSM (P<0,05), o virginiamicina maior GMD (P>0,05) e melhor eficiência financeira. As medidas biométricas não apresentaram diferenças (P>0,05), sugerindo que haja uma mudança na composição do GMD. Não houve diferença (P>0,05) para DISMS, DISFDA, pH ruminal e N-amoniacal. A virginiamicina apresentou as maiores degradabilidade efetiva da FDN (P>0,05) nas taxas de passagem de 2 e 5%, e semelhante ao controle e maior que salinomicina na taxa de 8%/hora. A virginiamicina ou a salinomicina, podem ser veiculados ao SM, contudo não promovem efeitos significativos no pH ruminal, no N-amoniacal, na DISMS e da DISFDA, mas a virginiamicina promoveu maior degradabilidade efetiva da FDN.
Palavras-chave: antimicrobianos, degradabilidade, crescimento, pH ruminal, N-amoniacal

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Introduction

The rumen is the most studied organ regarding the symbiotic interaction between host and microbiota in the digestive tract of ruminants. It may be the organ of greatest metabolic adaptability due to the presence of large and complex pre-gastric microflora. Thus, it plays a vital role in nutritional and physiological functions; besides, along with the intestines, it performs protective and immune functions in the host.

Virginiamycin is a cyclic polypeptide antibiotic combination, a product from the fermentation of several species of *Streptomyces* sp., of which *Streptomyces virginiae* is the main one. It consists of two main components: virginiamycin factor M1 and virginiamycin factor S1, which are synergistic and have microbial activity. This non-ionophore antibiotic acts by binding with ribosomes, inhibiting protein synthesis of certain microorganisms(1).

The salinomycin fits into another category of additive. It is classified as an ionophore additive, which is a polyesters carboxylic antibiotic; it is a product of the fermentation of various *Streptomyces* sp. strains. They are considered lipophilic substances, which are toxic to many bacteria and protozoa. By being a substance that acts in the selection of microorganisms, depressing or inhibiting their growth, it is used in ruminant nutrition with the aim of improving performance(2).

In view of the foregoing, the present experiment was conducted with the purpose of evaluating the inclusion of the additives virginiamycin and salinomycin to the mineral supplement for beef cattle fed tropical grass based diet during the rainy period. We evaluated supplement intake, weight gain performance, biometric measurements, and body condition score. Besides, the economic impact of this inclusion on growing beef cattle and its effects on ruminal pH, we also evaluated ammoniacal nitrogen concentrations and *in situ* degradability of dry matter (DM), neutral detergent fiber (NDF)

and acid detergent fiber (ADF).

Material and Methods

To perform this experiment, the research project was submitted to the Ethics Committee of the Universidade Federal de Goiás (UFG-COEP). The project was approved and registered under the protocol number COEP-UFG 039/11. The methods and procedures used in this study were performed according to the standards of the Brazilian College of Animal Experimentation (COBEA).

Three treatments were set: control treatment, mineral supplement only (COMIGO – CRIA 61 - F.2) (MS); virginiamycin treatment, MS plus virginiamycin (PhiGrow® - 20g/kg); and salinomycin treatment, MS plus salinomycin (Posistac® - 18g/kg).

Experiment 1

The research was conducted at the Experimental area for Livestock of the Technology Center COMIGO (CTC). A total of 45 intact Nellore steers were used, with initial weight of 239.3 kg (CV 20.45%), aged 13.5 months (± 1.5 months), and body condition score of 5.0 (CV 11.9%), from the same contemporary group.

The animals had access to massaigrass pasture (*Panicum maximum* cv. MASSAI) from 14 p.m. to 10 a.m. the next day and *ad libitum* access to water. Supplementation occurred in separate stalls for each treatment. The animals were enclosed from 10 a.m. to 14 p.m. for supplement intake with *ad libitum* access to water.

The forage samples were performed by means of "cage exclusion" (1 m^2), adapted from the model proposed by Diavão et al.[3]. It showed forage availability of 2,680 kg of dry matter/hectare ($DM.ha^{-1}$) in the grazing extract with DM (dry matter) content of 30.2 to 34.5%, CP (crude protein) from 11.2 to 11.8% of DM and estimate TDN (total digestible nutrients) of 57.1 to 60.2% of DM.

The animals were randomly distributed into a completely randomized design for performance evaluation. Treatments were arranged in three groups with 15 replicates (animals) each, whose mathematical model is: $y_{ij} = m + t_i + b_j + e_{ij}$, where: $\boldsymbol{y_{ij}}$ equals the observed value in the j^{th} experimental unit, which received the i^{th} treatment; $\boldsymbol{m}$ means the overall mean; $\boldsymbol{t_i}$ equals the i^{th} treatment effect; $\boldsymbol{b_j}$ is equivalent to the effect of the j^{th} block; $\boldsymbol{e_{ij}}$ is the experimental error associated with the observation $\boldsymbol{y_{ij.}}$ The collected data were statistically analyzed with the aid of the Statistical Analysis System[4], with means compared by Tukey test (P>.05).

The following aspects were evaluated: average daily supplement intake (MSI in g/animal/day), estimated by the difference between the quantity supplied and the remains in the trough; average daily gain (ADG in kg/animal/day) by individual weighing after a 15-hour fasting period; biometric measurements of anterior height (withers height), posterior height (hip height), body length, heart girth, hip width, chest width (adapted from Freneau et al.[5]) performed with the use of metric tape and hypometric cane; and body condition score (BCS) in the range 1-9, where 1 = extremely thin animal and 9 = obese animal[6].

The costs of including additives obtained by the proportional price of each additive in their formulation were quantified. The revenue from the weight gain of the animals was also quantified, which was obtained by average daily gain in weight and market price of living-kg weight.

Experiment 2

The research was conducted in the experimental area for beef cattle, Animal Production Department – Veterinary and Animal Science School / Universidade Federal de Goiás (EVZ / UFG). Six intact Nellore steers fistulated in the rumen from the same contemporary group were used. The animals were managed in the rainy season as in the Experiment 1, with forced ingestion of supplement leftovers via cannula to the predicted consumption of mineral mixture.

The pasture area was composed of grass paddocks (*Brachiaria decumbens*). The forage samples were performed by means of "cage exclusion" (1 m^2), adapted from the model proposed by Diavão et al.[3]. It showed minimum forage availability of 5,207.5 kg DM.ha^{-1} in the grazing extract, with average content of 39.3% DM.

A 3x3 (x2) Latin Square Outline was used, where each animal was randomly selected for treatment and period. Its mathematical model is: $y_{ijk} = m + \alpha_i + t_i + \beta_{k(ij)} + e_{ijk}$; where: $\boldsymbol{y_{ijk}}$ equals the observed value the j^{th} experimental unit, in the i^{th} period that received the k^{th} treatment; $\boldsymbol{m}$ means the overall average; $\boldsymbol{\alpha_i}$ is equivalent to the effect of the i^{th} period (row); $\boldsymbol{t_i}$ equals the effect of the j^{th} experimental unit (column); $\boldsymbol{\beta_k}$ equals the effect of the k^{th} treatment; $\boldsymbol{e_{ijk}}$ is the experimental error associated with the observation $y_{ij.}$ The collected data were statistically analyzed with the aid of the Statistical Analysis System[4], with means compared by Tukey test ($P > 0.05$).

The average daily consumption was evaluated by estimating the difference between the quantity supplied and the remains in the trough. For pH determination, a portable digital pH-meter was used (adapted from Cabral et al.[7]). To determine the concentration of ammoniacal nitrogen (mg N-NH3/dL), the methodology proposed by Chaney and Marbach[8] was uded. The samples of ruminal fluid were made by ruminal fistula at time 0 (zero) immediately before the energy-protein supplement being offered to animals and at 2, 4, 6 and 8 hours after the beginning of supplementation.

To test ruminal degradability we used nylon bags (poly-amide) with a porosity of 50 microns and contact area of 225 cm^2 (15 x 7.5 cm, both sides). The bags were incubated for 12 days, being 144, 120, 96, 72, 48, 24, 12, 8, 6, 4, 2 hours and 0 time (zero) to remove, adapted from the methodology proposed by Casali et al.[9]. The material incubated was *Brachiaria decumbens* hay, collected at the grazing area. The parameters were adjusted according to the equation proposed by Ørskov and McDonald[10]. After dry matter digestibility analysis (DMDIS), neutral detergent fiber digestibility (NDFDIS) and acid detergent fiber digestibility (ADFDIS) were evaluated by the serial method[11,12].

Analyses were processed with the aid of the program FitCurve6 for Windows® XP[13] to calculate the ruminal degradability, where potential degradation; lag time (time of microbial colonization); degradation of fractions "a" (rapidly soluble fraction), "b" (potentially degradable fraction) and "c" (constant degradation rate); effective degradability and water soluble degradability are evaluated.

Results and Discussion

In Experiment 1 (Table 1), the control group showed higher mineral supplement intake (MSI) in g/animal/day, compared to groups supplemented with MS plus virginiamycin and salinomycin (P> 0.05). The group virginiamycin showed a 18.7% reduction in supplement intake, while the salinomycin group had a 29.0% reduction compared to the control treatment. The reduction of MSI for salinomycin group was of 12.7% compared to the virginiamycin group. The control group showed lower amplitude of supplement intake.

The group virginiamycin showed a statistically (P> 0.05) higher ADG, 0.583 kg/animal/day; however, being higher than in the control group there was 25.4% increase, and in group salinomycin there was 9.79% increase. The group salinomycin showed ADG the 0.531 kg/animal/day; with 14.2% without ADG higher than the control group.

Table 1. Mean values of supplement intake and performance in the rainy season (Experiment 1)

Variables	Treatments			SE	P
	Control	Virginiamycin	Salinomycin		
MSI (g/animal/day)	45.5 [a]	37.0 [b]	32.3 [b]	1.62	0.0015
AdI (mg/animal/day)	0	74	69.77	-	-
Initial PV (kg)	238.0	240.6	239.2	-	-
Final PV (kg)	296.7 [a]	314.1 [b]	306.2 [ab]	0.03	0.0019
ADG (kg/animal/day)	0.465 [a]	0.583 [b]	0.531 [ab]	0.03	0.0019

Means on the same row do not differ statistically (P> 0.05).
SAS(4) statistical program for comparison of means by Tukey test.
SE – standard error.
P – value of the variable comparinson between treatments.
MSI – mineral supplement intake.
AdI – additive intake.

For animals reared under grazing, the results have shown considerable variation regarding performance and supplement intake. The animals that had virginiamycin and salinomycin added to the supplementation showed higher ADG, even with a lower supplement intake, which reflects directly on total weight gain (TWG). This makes clear that the use of antimicrobial additives have the potential to improve steers' performance.

The performance of grazing cattle depends on factors not fully characterized yet. Besides, several factors interfere with the response to the use of additives. Studies on zebu cattle under grazing of tropical grass with distinction between dry and rany seasons are even more scarce, although it represesnts the most common situation in Brazil.

The antimicrobial additives used in low-intake supplements tend to have lower daily intake, higher intake variability per animal and per animal per day during the period of use. Thefore, the addition of a supplement containing antimicrobial additive should be monitored and the salt content adjusted to obtain the desired consumption.

Biometric measurements showed no difference (P> 0.05) among treatments. However, weight gain (kg/animal/day) presented some differences, pointing to the need of finding new information about the relation of the use of virginiamycin and salinomycin and this aspect.

The use of antimicrobial additives suggests a change in the composition of weight gain among different tissues. According to Ferreira et al.[14], several factors may interfere with the chemical composition (protein, ether extract, water, and mineral matter), proportion and site of deposition of muscle and bone tissues, the proportion and site of deposition of adipose tissue (subcutaneous, intermuscular, perirenal, and mesenteric), as well as the non-carcass components. Some of these important factors are related to the nutritional actions on animal metabolism.

By analyzing the economic aspecs of inclusing additives (Table 2), the salinomycin group showed the greatest reduction in the cost of mineral supplementation, followed by the group treated with virginiamycin. However, the effect of the inclusion of additives in the mineral supplement on weight gain showed that the virginiamycin group, despite having higher daily cost, was the treatment with the greatest financial efficiency: 26% higher than the control treatment and 8.6% higher than the salinomycin treatment that was 16% higher than the control treatment.

Table 2. Economic aspects of the inclusion of the additives virginiamycin (VM) and salinomycin (MS) to the mineral supplement COMIGO (MSC*)

Variables	Treatments		
	Control	VM	MS
**MSI (Kg/animal/day)	0.0455 [a]	0.037 [b]	0.0323 [b]
***Cost/ kg MS (R$)	0.988	1.284	1.056
Cost/animal/ day(R$)	0.0450	0.0475	0.0341
ADG (kg/animal/day)	0.513 [a]	0.644 [b]	0.589 [ab]
*** Gross daily revenue (R$)	1.57	1.98	1.81
Abatement of supplementation (R$)	1.53	1.93	1.78
Economic efficiency (%)	Base 100	126	116

Means followed by the same letter on the same row do not differ statistically (P> 0.05).

*MSC - Mineral Supplement COMIGO, CRIA 61-F.2.

** MSI - mineral supplement intake.

*** Market values updated in Jan/2011 (R$ 3.07/kg live).

When the opportunity cost is higher than the feeding cost, the supplementation of cattle on grazing to optimize animal performance improve the annual net margin activity, which was verified with the use of virginiamycin and salinomycin. It is important to consider that the dietary practices that best apply to a particular situation are the ones that promote the highest biological efficiency coupled with maximum economic efficiency.

Therefore, the understanding the changes that additives produce in ruminal metabolism plays a very important role in animal production. There was no difference (P> 0.05) for mineral supplement intake in Experiment 2. For all treatments, mineral supplement intake was reduced from 28.6 to 39.5% compared to the predicted intake of 50 g/animal/day, the effect being more pronounced in animals fed diets with virginiamycin.

The results indicate that this intake reduction may have been influenced by the management of animals, which had already undergone surgery for cannulation and were submitted to constant manipulation of ruminal cannula and changes in the experimental area during the experiment. The stress of handling and restricted time for supplementation are likely to be the causes of the decrease in mineral supplement intake by the animals from this experiment[14].

Even with the predicted mineral supplement intake being met by forced ingestion, there was no

significant difference (P> 0.05) on ruminal pH. All treatments showed an optimal range of ruminal pH, with 6.66 (control), 6.61 (virginiamycin), and 6.56 (salinomycin) for the proper rumen functioning.

The fiber digestion rate can be reduced by the low ruminal pH, since this also causes changes in the growth rate of microorganisms affecting the proportion of microbial species in the rumen; also the cellulase activity is affected when the pH is below 6.0. However, the reduction to values of 5.5 to 5.0 results in decline in growth rate and reduction of fibrolitic microorganisms and fiber digestion can be inhibited[15].

Ruminal pH is affected by the type of food consumed and its stabilization is attributed in large part to the saliva, which has high buffering capacity[16]. The saliva undergoes an increase in its flow due to the stimulation of chewing and rumination, which results from reflections started by physical stimuli of coarse particles on the ruminal wall. Thus, the action of ruminal manipulators has its effect reduced or canceled for this parameter, as happened in this study, since the animals had their forage-based diet with mineral supplement.

Similarly to ruminal pH, the concentration of ammoniacal nitrogen in the rumen may compromise the activity of ruminal microorganisms, especially those that degrade fibrous carbohydrates, which is dependent on the level of ammoniacal nitrogen in the rumen. The use of selector additives of ruminal microbiota aims at promoting adequate levels of ammoniacal nitrogen to microbial growth.

The concentration of ammoniacal nitrogen (in mg $N\text{-}NH_3$/dL) during the rainy season showed no statistical differences (P> 0.05) among treatments, with means of 3.9 (salinomycin) and 4.2 (virginiamycin and control).

Knowledge of the imbalance in the digestion of protein can contribute for the determination of ammoniacal nitrogen concentrations in the rumen, since high ammoniacal nitrogen concentrations may indicate an excess of degraded dietary protein in the rumen and/or low degraded carbohydrate concentration in the rumen[17]. The concentration of ammoniacal nitrogen in the rumen depends on the balance between production rates and their use. For maximization of ruminal dry matter digestion, maximizing consumption in tropical conditions concentrations of 3.3 and 8.0 mg $N\text{-}NH_3$/ dL are required[14], range of the results found in this.

Besides ruminal pH and amoniacal nitrogen concentration, other factors affect ruminal digestion and nutrients absorption such as the degradability of the content ingested. In the case of animals under grazing or fed diets with high portion of bulkyforage, dry matter and fibers degradability should be highlighted.

The average values determined for DMDIS under the conditions of this study showed no significant difference (P> 0.05) for any variable.

Tropical grasses have limitations regarding the qualitative aspect with reduced cellular content and high deposition of content in the cell wall. The fiber fraction generally has slow and incomplete digestion, being the primarily responsible for the variation in the food digestion. Thus, manipulation of ruminal microbiota with the use of antimicrobial additives or microbiota selectors will have little effect on the degradation of the content ingested when it has particles of considerable size, as demonstrated in this work because the animals grazed on tropical pasture and received no processed-based diet.

There were differences (P> 0.05) for the effective degradation in the three passage rates analyzed for *in situ* degradability of NDF (Table 3). Treatment with virginiamycin had the highest degradability in passage rates of 2 and 5%, which was similar to control group and greater than salinomycin in the rate of 8%/hour. Control and salinomycin treatments did not differ in passage rates during the rainy season for NDFDIS. There were no differences (P> 0.05) for the remaining parameters in the rainy season for NDFDIS.

Table 3. Mean values of *in situ* degradability of neutral detergent fiber (NDFDIS) during rainy season

	Treatments				
Variables	**Control**	**Virginiamycin**	**Salinomycin**	**SE**	**P**
Potential degradation (%)	53.43	42.42	78.70	41.5	0.1904
Lag Time (h)	7.75	3.98	4.22	53.5	0.5718
Adjusted parameters					
Fraction "a"	19.97	18.85	20.53	8.80	0.5827
Fraction "b"	70.42	23.53	119.87	27.8	0.2203
Fraction "c"	0.012	0.041	0.002	0.01	0.1256
Effective degradability					
2 % / hour	23.77 [b]	26.37 [a]	23.37 [b]	31.8	0.0141
5 % / hour	22.02 [b]	24.12 [a]	21.78 [b]	19.8	0.0162
8 % / hour	21.45 [ab]	23.10 [a]	21.36 [b]	11.5	0.0292
Water Soluble Digestibility (%)	33.45	21.88	58.28	41.5	0.1840
Losses in Washing (%)	19.98	20.50	20.42	0.92	0.6568

Means followed by the same letter on the same row do not differ statistically (P> 0.05).
SAS statistical program[4] for comparison of means by Tukey test.
SE – standard error.
P – value of the variable comparinson between treatments.

The average values of effective degradability for a passage rate of 2% and 5% per hour represent diets with higher portion of forage. The passage rate of 2% per hour corresponds to diets rich in foods with high FDA concentration, i.e. low-quality tropical forages; and that of 5% per hour is for higher quality forage diets, i.e. pastures with low concentrations of lignin[18].

Food digestibility means the ability of the food to allow the animal to use their nutrients in a greater or lesser extent. This ability is expressed by the coefficient of degradability of the nutritional components of interest. Digestibility and intake are the two main components that determine the nutritional value of a food. Of all the nutrients required for cattle maintenance, growth and/or production, the energy from the ruminal degradation of cellulose, hemicellulose and lignin (the main constituents of neutral detergent fiber) constitutes the main contribution of the forage. According to Van Soest[16], the NDF content of the food is inversely related to the voluntary consumption of food.

For acid detergent fiber there were no statistical differences (P> 0.05) for any of the parameters analyzed. The acid detergent fiber is the portion with the lowest digestibility in the cell wall. It consists of lignocellulose (cellulose and lignin) being inversely proportional to digestibility: the higher the percentage of acid detergent fiber the lower the material digestibility according to Van Soest[16].

The increase in growth efficiency caused by the use of selector additives of ruminal microbiata can

sometimes be explained by the increased digestibility of food constituents, especially the dry matter and fibers in the case of tropical grasses. However, it is necessary that other variables are evaluated simultaneously, since it is possible to bring other benefits to the use of antimicrobial additives without any changes in certain parameters.

The addition of virginiamycin to mineral supplement improved the performance of cattle under grazing, without influencing the biometric measurements. Still, it reduced the consumption of mineral supplement, improved economic efficiency and reduced the cost of mineral supplementation for cattle under grazing.

Conclusions

The concentration of ammoniacal nitrogen, ruminal pH, degradability of DM and ADF were not affected by the addition of virginiamycin and salinomycin to the mineral supplementation of cattle under grazing. However, virginiamycin improved the effective degradability of NDF.
Further research is recommended to better understand the actions of the molecules studied, and inclusion levels of the molecule in supplements for cattle under grazing on tropical grasses.

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