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Effects of antibiotic growth promoters and concentrate on intake, digestibility, degradability, and ruminal variables in beef steers

[*Efeitos de antibióticos promotores de crescimento e do concentrado sobre o consumo, digestibilidade, degradabilidade e variáveis ruminais de novilhos de corte*]

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ABSTRACT

The objective of this study was to evaluate the addition of antibiotic growth promoters to concentrate on intake, digestibility, *in situ* degradability and ruminal variables in steers. Four steers in a Latin square design were fed hay (*ad libitum*), concentrate [10g kg^{-1} of body weight (BW)] and mineral mix (0.272g kg^{-1} of BW). The additives (0.75mg kg^{-1} of BW) were incorporated in the mineral mix as follows: Control (no antibiotics), lasalocid (LASA), salinomycin (SALI) or virginiamycin (VIRG). Antibiotic did not affect intake, pH, volatile fatty acids (VFA) and digestibility of dry matter (DM), organic matter, crude protein, ethereal extract, and non-fibrous carbohydrates. The LASA and SALI tended (P=0.09) to reduce the digestibility of neutral detergent fiber (NDF). The SALI and VIRG tended (P=0.09) to reduce the DM disappearance, and VIRG tended (P=0.06) to reduce the NDF disappearance in the rumen. The SALI and VIRG reduced the effective degradation and only SALI reduced the concentration of N-NH_3 in the rumen. Thus, the antibiotics did not affect intake, pH, VFA and digestibility, but decreased the degradation of fiber and only SALI reduced the concentration of ammonia nitrogen (N-NH_3) in the rumen.

Keywords: additives, antibiotics, growth promoters, ionophores, ruminants

RESUMO

O objetivo foi avaliar a adição de antibióticos promotores de crescimento ao concentrado sobre o consumo, digestibilidade, degradabilidade in situ *e variáveis ruminais de novilhos. Quatro novilhos em um delineamento quadrado latino foram alimentados com feno (*ad libitum*), concentrado [10g kg^{-1} do peso corporal (PC)] e mistura mineral (0,272g kg^{-1} do PC). Os antibióticos (0,75mg kg^{-1} do PC) foram incorporados à ração: Controle (sem antibióticos), lasalocida (LASA), salinomicina (SALI) ou virginiamicina (VIRG). Os antibióticos não afetaram o consumo, pH, ácidos graxos voláteis (AGV) e digestibilidade da matéria seca (MS), matéria orgânica, proteína bruta, extrato etéreo e carboidratos não fibrosos. A LASA e a SALI tenderam (P=0,09) a reduzir a digestibilidade da fibra em detergente neutro (FDN). A SALI e a VIRG tenderam (P=0,09) a reduzir o desaparecimento da MS, e a VIRG tendeu (P=0,06) a reduzir o desaparecimento da FDN no rúmen. A SALI e a VIRG reduziram a degradação efetiva e somente a SALI reduziu a concentração de nitrogênio amoniacal (N-NH_3) no rúmen. Assim, os antibióticos não afetaram o consumo, pH, AGV e digestibilidade, mas diminuiram a degradação da fibra e somente a SALI reduziu a concentração de N-NH_3 no rúmen.*

Palavras-chave: aditivos, antibióticos, ionóforos, promotores de crescimento, ruminantes

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INTRODUCTION

Ionophores, such as lasalocid (LASA) and salinomycin (SALI), are most used as growth promoting additives for beef cattle and are widely researched and used (Page, 2003). Virginiamycin (VIRG) belongs to the streptogramin class and has a different mechanism of action on microorganisms in the rumen (ribosome function inhibitors) compared to ionophores (cation disruptors; Page, 2003). Growth promoting additives beneficially alter the bacterial population in the rumen by acting on Gram-positive bacteria, enabling better conditions for Gram-negative bacteria to develop (Pressman, 1976). These alterations in the rumen's microbiology are reflected in the lower production of methane, ammonia, acetic and butyric acids, and greater production of propionic acid (Russel and Strobel, 1989; Nutrient…, 2000; Vargas *et al*., 2001; Golder and Lean, 2016). Thus, this alteration in the rumen improves the efficiency of protein and energy utilization of the diet (Page, 2003; Russel and Strobel, 1989) and may result in higher weight gain in beef cattle (Bretschneider *et al*., 2008). Besides that, growth promoting additives can act on cellulolytic bacteria (Mcallister *et al*., 1994), which can lead to a reduction in fiber degradability in diets with high roughage inclusion.

LASA, SALI and VIRG do not act on the same ruminal microorganisms and the intensity of action may be different for each additive in each microorganism (Page, 2003), therefore, ruminal fermentation can be modified differently by each additive. However, we are unaware of studies comparing the effects of LASA, SALI and VIRG on intake, digestibility, degradability, and ruminal variables in beef cattle. In addition, most studies evaluating the inclusion of growth promoting additives were tested in diets with high concentrate inclusion and few in predominantly roughage diets (Vargas *et al*., 2001; Golder and Lean, 2016). Further, we hypothesize that when LASA, SALI and VIRG are included in diets primarily consisting of roughage, they will impair fiber degradation due to the effect on cellulolytic bacteria. Thus, the objective of this study was to evaluate the effects of LASA, SALI and VIRG on intake, digestibility, in situ degradability and ruminal variables (pH, N-NH3 and VFA) in Nellore steers.

MATERIAL AND METHODS

The experiment was conducted according to the institutional Ethics Committee on Animal Use (number 366/2011).

Four cannulated Nellore (*Bos indicus*) steers with initial body weight (BW) of 438 ± 39kg were used in this study. Steers were housed in individual pens in a covered shed with adequate ventilation. Pens had concrete floors, feeder and drinking fountain. Steers were fed with chopped Massai-grass hay [*Megathyrsus maximus (Jacq.)* Simon & Jacobs], concentrate and mineral mix (Table 1). The concentrate formulation contained fine ground corn (910g kg^{-1}), soybean meal (65g kg^{-1}) and urea (25g kg^{-1}). Hay and concentrate were provided in two daily meals at 0700 h and 1700 h. Mineral mix was offered only at 0700 h and was mixed with concentrate to ensure that all mineral mix was consumed. Mineral mix was utilized as a vehicle to provide steers with the following treatments: Control (no addition of growth promotors), LASA, SALI and VIRG. Hay was offered ad libitum, while concentrate was offered at 10g kg^{-1} of BW, mineral mix at 0.272g kg^{-1} of BW and additives at 0.75mg kg^{-1} of BW. The dosage of additives used was based on the meta-analysis of Bretschneider *et al*. (2008). Mineral mix was produced and packaged by a mineral supplementation company that added antibiotic growth promoters to a commercial formula (Table 1).

The experimental design was a 4 x 4 Latin square. Four experimental periods of 28 days each were performed as 21 days of adaptation to treatment and 7 days of data collection. At the initiation of each new experimental period, animals were weighed after a 16-hour removal from feed to adjust the amount of concentrate and mineral mix/additives.

Table 1. Chemical composition of concentrate, Massai-grass hay [*Megathyrsus maximus (Jacq.)* Simon & Jacobs] and guaranteed levels of mineral mix

Items	Chemical composition (g kg^{-1} DM)			
	Concentrate	Hay		
Dry matter (DM)	860.00	880.00		
Organic matter (OM)	981.80	953.20		
Crude protein (CP)	184.70	36.00		
Neutral detergent fiber (NDF)	169.50	788.30		
Ethereal extract (EE)	35.80	9.750		
Non-fibrous carbohydrates (NFC)	620.52	104.42		
Ashes	18.20	46.80		
Items	Guaranteed levels of mineral mix[1]			
	Control	LASA	SALI	VIRG
Calcium (g kg^{-1})	194	184	187	185
Sulfur (g kg^{-1})	16	16	16	16
Phosphorus (g kg^{-1})	70	70	70	70
Sodium (g kg^{-1})	109	109	109	109
Cobalt (mg kg^{-1})	50	50	50	50
Copper (mg kg^{-1})	1050	1050	1050	1050
Iodine (mg kg^{-1})	60	60	60	60
Manganese (mg kg^{-1})	900	900	900	900
Selenium (mg kg^{-1})	12	12	12	12
Zinc (mg kg^{-1})	3500	3500	3500	3500
Lasalocid (g kg^{-1})	-	2750	-	-
Salinomycin (g kg^{-1})	-	-	2750	-
Virginiamycin (g kg^{-1})	-	-	-	2750

[1]Control, no antibiotic growth promoters; LASA, lasalocid; SALI, salinomycin; VIRG, virginiamycin.
Hay was offered *ad libitum*, concentrate in the amount of 10g kg^{-1} of body weight (BW), mineral mix of 0.272g kg^{-1} of BW and antibiotic growth promoters 0.75mg kg^{-1} of BW.

Daily intake was determined between day 22 and 26 of each experimental period by weighing the amount of feed offered and orts. Feces were collected in the same period and all pens were washed on day 21 and kept clean to avoid any fecal contamination during the collection period. All feces were collected (24 hours per day) and allocated in a bucket identified per animal. Every 6 hours, the amount of feces per animal was weighed, homogenized, and samples equivalent to 100g kg^{-1} were stored (-20ºC) for further analysis. Based on this information, the following variables were evaluated: intake (offered – orts), apparent digestibility coefficients [(nutrient intake – excreted nutrient)/nutrient intake] of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), ethereal extract (EE), and non-fibrous carbohydrate (NFC). The total digestible nutrients (TDN) were estimated by the formula proposed by Sniffen *et al*. (1992): TDN = digestible CP + digestible NDF + (2.25 x digestible EE) + digestible NFC.

Ruminal degradability of DM and NDF was determined from day 22 to 26 of each experimental period using 7 x 14cm nylon bags with a porosity of 50μm, sealed at the edges and properly identified. Nylon bags were weighed empty, filled with 5g of hay (ground and passed through a 2mm pore sieve), and tied with a rubber band to a metal ring to keep them closed. Bags were first soaked in water for one hour and subsequently attached to a metal chain of 50cm with an anchor weighing approximately 600g. Bags were then infused into the rumen at 0700h (before feeding) and removed after the incubation times (3, 6, 12, 24, 48, 72, 96 and 120h). After removal from the rumen, bags were immediately immersed in ice water and washed in a washing machine for five minutes for three cycles, with new water for each cycle. Bags were dried in a forced air ventilation oven at 55ºC for 72 hours and weighed.

The DM soluble fraction was determined by placing a sample of hay in a nylon bag and placing bags in water (38°C) for one hour. Following, bags were washed in a washing machine, oven dried, and weighed. The difference between the initial and final weight was considered as the soluble fraction for each

experimental period, which corresponds to the value at 0 h in the DM degradation curve. The soluble fraction "a", insoluble fraction "b", rate of degradation "c", and effective degradability (ED) were calculated according to Ørskov and Mcdonald (1979) with the equation ED = a + (b x c) / (c + k), where "k" is the estimated rumen solids passage rate calculated as 0.02 and 0.05 h^{-1}.

Ruminal fluid samples were collected for the determination of volatile fatty acid (VFA), pH, and N-NH_3 from day 27 to 28 in each experimental period. Samples were collected at the end of the experimental period following removal of the nylon bags. At time of collection, samples were taken at zero hours (before supplementation) and at 2, 4, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours after the morning feeding and always following the same sequence among animals. The ruminal fluid collection was performed manually and filtered in cloth. An aliquot of approximately 100mL of ruminal fluid was collected. The pH was measured immediately after the collection of ruminal fluid using a digital potentiometer (B474; Micronal, São Paulo, SP, Brazil). Ruminal fluid for VFA analysis was prepared using 4mL of ruminal fluid acidified with 1mL of metaphosphoric acid (25%) and stored at -20°C. Preparation of N-NH_3 for analysis used 50mL of ruminal fluid acidified with 1mL of H_2SO_4 (50%) and stored at -20°C.

The chemical composition of hay, concentrate, orts and feces was conducted according to AOAC (Official…, 1990) as follows: DM - method 967,03; CP - method 981,10; ashes - method 942,05; and EE – method 920,29. The NDF content was analyzed in a Tecnal TE-149® fiber analyzer (Tecnal, Piracicaba, SP, Brazil) using 5 X 5cm non-woven fabric (NWF) bags with 100µm porosity. Samples (of feed or feces) were added to the bags at 0.5g and were analyzed for neutral detergent fiber according to the methodology of Van Soest *et al*. (1991) without sodium sulfite and amylase. The same procedure used for the NDF was used to analyze the material resulting from the *in situ* ruminal degradation. The NFC content was calculated as proposed by Sniffen *et al*. (1992) with the equation: NFC = 100 - (CP + ashes + NDF + EE).

The N-NH_3 analysis used the supernatant of ruminal fluid samples thawed at 4ºC and distillation with 2N KOH according to Ribeiro *et al*. (2011). The concentrations of VFA were determined by gas chromatography (Thermo Electron Corporation, Trace CG Ultra, Italy) according to the methodology described by Erwin *et al*. (1961).

All data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC; version 9.4) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The statistical model for intake, apparent digestibility, and the estimation of the ruminal parameters of DM and NDF was:

$$Yijk = \mu + Ti + Pj + Ak + \varepsilon ijk$$

Where: $Yijk$ = observation of the effect of treatment i in period j, of animal k, where μ is the overall mean; Ti = effect of treatment i, where i = 1 (Control), 2 (LASA), 3 (SALI) and 4 (VIRG); Pj = effect of period j (j = 4 periods); Ak = effect of animal k (k = 4 animals), and εijk = random error associated with each observation.

The statistical model for *in situ* degradation rate, pH, N-NH_3 and VFA was:

$$Yijk = \mu + Ti + Hj + Ak + Pj + (TH)ij + \varepsilon ijk$$

Where: $Yijk$ = observation of the effect of treatment i per hours of incubation (rate of degradation) or collection time (ruminal variables) j in animal k; μ = overall mean; Ti = effect of treatment (i = 1 (Control), 2 (LASA), 3 (SALI) and 4 (VIRG); Hj = effect of incubation hours for degradability (j = 1,....., 8) or collection times for ruminal variables (j = 1, ..., 13); Ak = animal effect (k = 1, ..., 4); Pj = the period effect (j= 1, ..., 4); $THij$ = interaction between treatment i and time j; and εijk = random error associated with each observation. The data were analyzed as a repeated measure and the first order autoregressive covariance structure was selected for all variables due the lowest Akaike information criterion.

Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$ and tendency when $P > 0.05$ and ≤ 0.10.

RESULTS

There was no treatment effect (P≥0.26) on intake of hay or total diet in kg day^{-1} or g kg^{-1} of BW (Table 2). Likewise, effect of treatment was not detected (P≥0.14) for apparent digestibility coefficients of DM, OM, CP, EE, NFC and TDN (Table 3). However, Control steers tended (P=0.09) to have greater apparent digestibility of NDF, compared to LASA and SALI steers, with VIRG steers being intermediate (Table 3).

Effects of treatment × hour tended to be detected (P=0.09) for DM disappearance in the rumen at 12, 48, 72 and 96h after incubation (Table 4). The Control and LASA steers tended to have greater DM disappearance in the rumen compared to VIRG steers, and SALI steers did not differ from other treatments. Effects of hour, but not treatment (P=0.22), were detected (P<0.01) for DM disappearance in the rumen with disappearance increasing until h 96; however, disappearance did not differ between 96 and 120 h after hay incubation (Table 4). Effects of treatment, but not treatment × hour (P=0.31) tended to be detected (P = 0.06) for NDF disappearance (Table 4). Control, LASA and SALI steers tended to have greater NDF disappearance compared to VIRG steers. Effect of hour was detected (P<0.01) for NDF disappearance and the disappearance increased until 96 h but did not differ between 96 and 120 h after hay incubation (Table 4).

Table 2. Effect of antibiotic growth promoters and concentrate on intake (kg day^{-1} and g kg^{-1} of BW) of DM, OM, CP, NDF, EE, NFC of hay and total diet (hay + concentrate) in steers

Intake of[1]	Treatments[2]				SEM	*P*-value
	Control	LASA	SALI	VIRG		
kg day^{-1}						
DM Hay	4.96	4.81	4.70	4.72	0.315	0.60
DM Total	8.94	8.73	8.66	8.66	0.363	0.60
OM Hay	4.70	4.55	4.45	4.47	0.304	0.59
OM Total	8.51	8.30	8.24	8.24	0.351	0.60
CP Hay	0.18	0.18	0.17	0.17	0.010	0.51
CP Total	0.90	0.88	0.89	0.89	0.038	0.26
NDF Hay	3.88	3.74	3.66	3.69	0.246	0.55
NDF Total	4.56	4.40	4.32	4.35	0.232	0.55
EE Feno	0.16	0.16	0.15	0.16	0.006	0.44
EE Total	0.38	0.37	0.36	0.37	0.016	0.51
Ashes Hay	0.27	0.26	0.26	0.25	0.018	0.72
Ashes Total	0.33	0.33	0.33	0.32	0.015	0.69
NFC Hay	0.47	0.47	0.46	0.45	0.087	0.82
NFC Total	2.83	2.79	2.81	2.79	0.173	0.81
g kg^{-1} of BW						
DM Hay	10.89	10.72	10.28	10.40	0.952	0.52
DM Total	19.51	19.32	18.88	19.02	0.908	0.50
OM Hay	10.30	10.13	9.72	9.85	0.909	0.53
OM Total	18.54	18.37	17.95	18.08	0.867	0.51
CP Hay	0.40	0.40	0.39	0.39	0.036	0.67
CP Total	1.97	1.96	1.95	1.94	0.056	0.39
NDF Hay	8.51	8.31	7.99	8.14	0.746	0.52
NDF Total	9.96	9.77	9.45	9.60	0.728	0.52
EE Feno	0.36	0.35	0.34	0.34	0.028	0.31
EE Total	0.82	0.81	0.80	0.81	0.039	0.49
Ashes Hay	0.58	0.59	0.56	0.56	0.054	0.37
Ashes Total	0.96	0.95	0.93	0.94	0.051	0.51
NFC Hay	1.03	1.07	1.00	0.97	0.196	0.40
NFC Total	6.13	6.16	6.08	6.06	0.174	0.49

[1]DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; EE, ethereal extract; NFC, non-fibrous carbohydrates.
[2]Control, no antibiotic growth promoters; LASA, lasalocid; SALI, salinomycin; VIRG, virginiamycin.

Table 3. Effect of antibiotic growth promoters and concentrate on the apparent digestibility coefficients of DM, OM, CP, NDF, EE and NFC in beef steers

Digestibility of[1]	Treatments[2]				SEM	*P*-value[3]
	Control	LASA	SALI	VIRG		
DM (fraction 0–1)	0.58	0.57	0.56	0.58	0.019	0.65
Digestible amount (g kg DM^{-1})						
OM	598.77	586.28	574.81	598.6	19.394	0.49
CP	683.72	699.71	674.14	702.58	26.003	0.54
NDF	425.17^{a}	364.23^{b}	337.93^{b}	378.05^{ab}	21.899	0.09
EE	906.83	905.51	932.65	908.66	14.567	0.14
NFC	763.15	721.17	777.22	744.70	46.302	0.76
TDN	571.46	580.57	573.02	599.53	23.285	0.55

[1]DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; EE, ethereal extract; NFC, non-Fibrous carbohydrates; TDN, total digestible nutrients.
[2]Control, no antibiotic growth promoters; LASA, lasalocid; SALI, salinomycin; VIRG, virginiamycin.
[3]Means followed by different letters in the same row showed a tendency to differ ($P \leq 0.10$).

Table 4. Effect of antibiotic growth promoters and concentrate on disappearance of dry matter (DM) and neutral detergent fiber (NDF) of hay in the rumen (expressed as a fraction of 0-1) in beef steers

Incubation hours	Treatments[1]				SEM	*P*-value[2]		
	Control	LASA	SALI	VIRG		Treatment	Hour	Treatment × Hour
	DM disappearance					0.22	<0.01	0.09
0	0.15^{H}	0.15^{H}	0.15^{H}	0.15^{H}	0.008			
3	0.21^{G}	0.21^{G}	0.21^{G}	0.20^{G}	0.008			
6	0.23^{F}	0.23^{F}	0.23^{F}	0.22^{F}	0.008			
12	0.27^{Ea}	0.28^{Ea}	0.26^{Eab}	0.24^{Eb}	0.008			
24	0.33^{D}	0.35^{D}	0.35^{D}	0.33^{D}	0.008			
48	0.44^{Cab}	0.45^{Ca}	0.44^{Cab}	0.43^{Cb}	0.008			
72	0.50^{Ba}	0.49^{Ba}	0.48^{Bab}	0.47^{Bb}	0.008			
96	0.53^{Aa}	0.53^{Aa}	0.51^{Ab}	0.50^{Ab}	0.008			
120	0.55^{A}	0.55^{A}	0.54^{A}	0.53^{A}	0.008			
	NDF disappearance					0.06	<0.01	0.31
3	0.17 ^{G}	0.16^{G}	0.16^{G}	0.14^{G}	0.009			
6	0.18^{F}	0.18^{F}	0.18^{F}	0.16^{F}	0.009			
12	0.21^{E}	0.23^{E}	0.22^{E}	0.20^{E}	0.010			
24	0.29^{D}	0.31^{D}	0.30^{D}	0.28^{D}	0.009			
48	0.40^{C}	0.42^{C}	0.39^{C}	0.39^{C}	0.009			
72	0.46^{B}	0.47^{B}	0.45^{B}	0.43^{B}	0.009			
96	0.50^{A}	0.50^{A}	0.48^{A}	0.47^{A}	0.009			
120	0.53^{A}	0.53^{A}	0.52^{A}	0.48^{A}	0.009			
Average	0.34^{a}	0.35^{a}	0.34^{a}	0.32^{b}	0.006			

[1]Control, no antibiotic growth promoters; LASA, lasalocid; SALI, salinomycin; VIRG, virginiamycin.
[2]Means followed by different lowercase letters in the same row or different capital letters in same columns, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

Treatment affected the estimation of ruminal variables for DM and NDF degradation of hay. Effect of treatment was not detected (P=0.28) for the potentially degradable insoluble fraction of DM (Table 5). The Control and LASA steers had greater (P=0.01) degradation rates of DM, compared to VIRG steers, but SALI steers did not differ from Control and VIRG steers. The Control and LASA steers had greater (P≤0.01) ED of DM compared to SALI and VIRG steers. The Control, LASA and SALI steers had a greater (P=0.02) potentially degradable insoluble fraction of NDF compared to VIRG steers. The LASA steers tended to have a greater (P=0.09) degradation rate of NDF compared to SALI steers, but Control and VIRG steers did not differ from the other treatments. The Control and LASA steers had greater ($P \leq 0.009$) ED of NDF, compared to SALI and VIRG steers (Table 5).

Table 5. Effect of antibiotic growth promoters and concentrate on the estimation of ruminal variables of dry matter (DM) and neutral detergent fiber (NDF) degradation of hay (values are expressed as the fraction of 0-1)

Parameters[1]	Treatments[2]				SEM	*P*-value[3]
	Control	LASA	SALI	VIRG		
DM (a=0.154)						
b	0.393	0.398	0.384	0.378	0.011	0.28
c	0.031^{ab}	0.035^{a}	0.027^{bc}	0.024^{c}	0.002	0.01
ED (0.02 h^{-1})	0.486^{a}	0.482^{a}	0.459^{b}	0.457^{b}	0.007	<0.01
ED (0.05 h^{-1})	0.364^{a}	0.358^{a}	0.339^{b}	0.338^{b}	0.008	<0.01
NDF						
b	0.527^{a}	0.526^{a}	0.516^{a}	0.495^{b}	0.012	0.02
c	0.028^{ab}	0.029^{a}	0.025^{b}	0.027^{ab}	0.001	0.09
ED (0.02 h^{-1})	0.462^{a}	0.464^{a}	0.439^{b}	0.438^{b}	0.005	<0.01
ED (0.05 h^{-1})	0.344^{a}	0.346^{a}	0.325^{b}	0.328^{b}	0.004	<0.01

[1]Soluble fraction, b: insoluble fraction potentially degradable, c: degradation rate (/h), ED: effective degradation (considering a degradation rate of 0.02 and 0.05 h^{-1}).
[2]Control, no antibiotic growth promoters; LASA, lasalocid; SALI, salinomycin; VIRG, virginiamycin.
[3]Means followed by different letters in the same row differ ($P \leq 0.05$) or show a tendency to differ ($P \leq 0.10$).

Effects of hour (P<0.01), but not treatment × hour or treatment (P≥0.20), were detected for ruminal pH (Figure 1). Rumen pH was greater 2-4 h after feeding and was lowest approximately 6-10h after feeding (Figure 1). Effects of treatment and hour, but not the treatment × hour interaction (P=0.41), were detected ($P \leq 0.04$) for NH_3-N in the rumen (Figure 2). The Control (13.76mg dL^{-1}) and VIRG (12.72mg dL^{-1}) steers had a greater concentration of NH_3-N in the rumen, compared to SALI (10.48mg dL^{-1}) steers, but LASA (11.85mg dL^{-1}) steers did not differ from the other treatments (Figure 2).

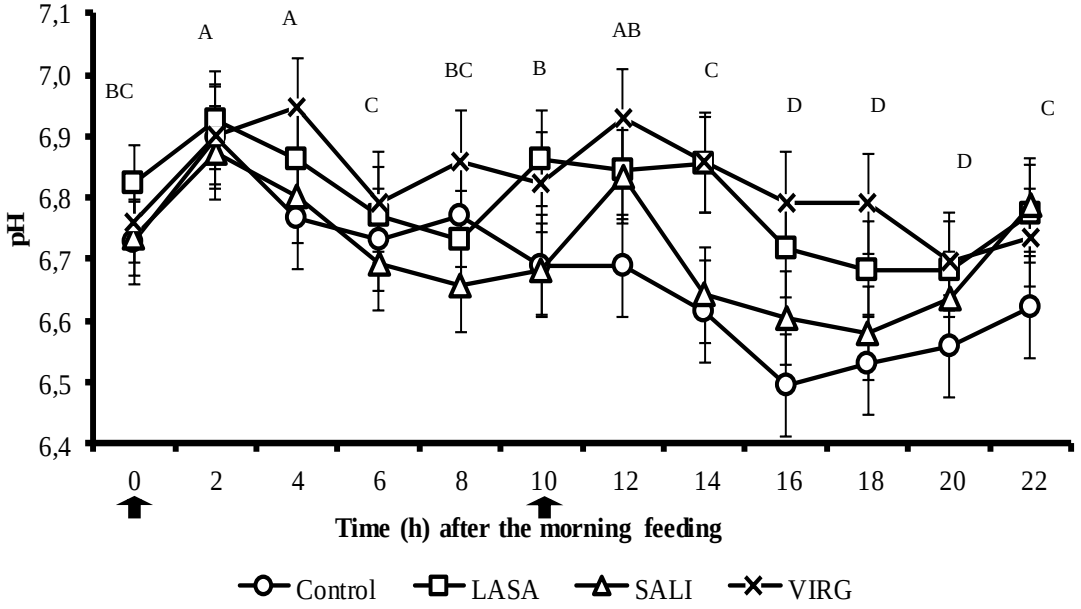

Figure 1. Mean values of pH in the rumen of steers at different collection times receiving the following treatments: Control, no antibiotic growth promoters; LASA, lasalocid; SALI, salinomycin; VIRG, virginiamycin. Arrows represent the time of diet supply. Different capital letter represents statistical differences (P≤0.05) between hours. Vertical bars represent the standard deviation. Effects of hour, but not treatment × hour and treatment (P≥0.20) were detected (P<0.01) for ruminal pH.

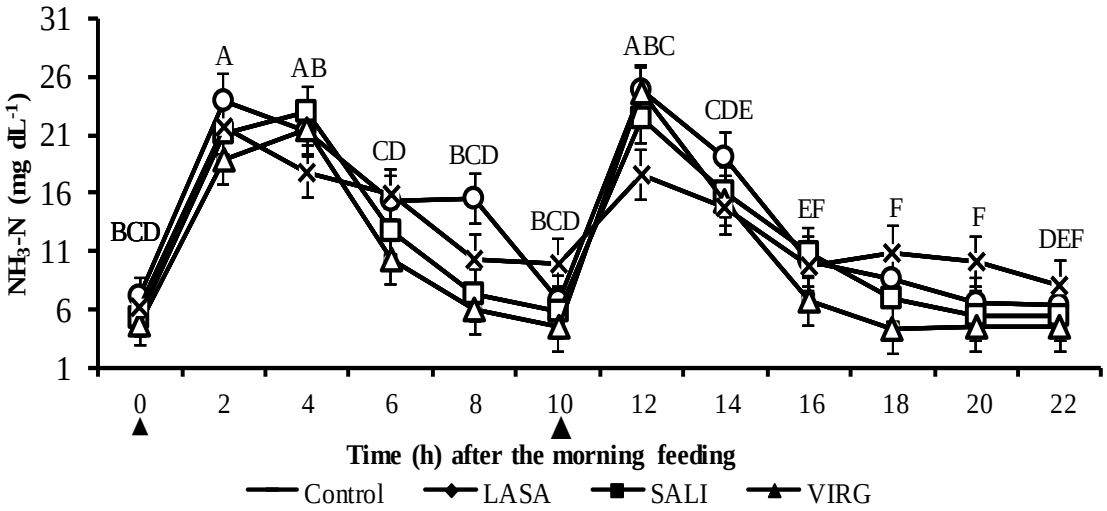

Figure 2. Mean values of ammonia nitrogen (NH_3-N) in the rumen of steers at different collection times receiving the following treatments: Control, no addition of growth promoters; LASA, lasalocid; SALI, salinomycin; VIRG, virginiamycin. Arrows represent the time of diet supply. Vertical bars represent the standard deviation. Different capital letters mean that hours differ (P≤0.05). Effects of treatment and hour, but not interaction between treatment × hour (P=0.4108), were detected (P≤0.04).

Effects of treatment × hour and treatment were not detected (P≥0.11) for volatile fatty acids (VFA) in the rumen (Table 6). Effect of hour was detected (P≤0.05) in mmol L^{-1} for acetate, propionate, butyrate, N-butyrate, valerate, isovalerate, N-valerate, total VFA and the acetate: propionate ratio, and tended to be detected (P=0.08) for isobutyrate (Table 6; Figure 3). Effect of hour was detected (P = 0.004) in mmol $100mmol^{-1}$ only for acetate (Table 6). Total VFA, acetate, propionate, butyrate and valerate concentrations (mmol L^{-1}) were greater around 6 h after feeding and the lowest concentrations occurred at time of feeding. The greatest acetate: propionate ratio occurred at time of feeding, while the lowest ratio was observed 6 h after the afternoon feeding (Figure 3).

Table 6. Effect of antibiotic growth promoters and concentrate on the production of volatile fatty acids (VFA) in the rumen of steers

Items	Treatments[1]				SEM	*P*-value[2]		
	Control	LASA	SALI	VIRG		Treatment	Hour	Treatment × Hour
VFA (mmol L^{-1})								
Acetate	68.30	62.77	66.07	65.90	3.25	0.78	<0.01	0.27
Propionate	13.68	12.96	12.84	11.84	1.09	0.70	<0.01	0.90
Butyrate	12.63	11.19	11.87	12.36	0.83	0.77	<0.01	0.81
Isobutirate	0.63	0.58	0.61	0.64	0.07	0.95	0.08	0.87
N-butirate	12.07	10.64	11.26	11.78	0.79	0.75	<0.01	0.77
Valerate	1.27	1.13	1.19	1.25	0.13	0.86	0.05	0.98
Isovalerate	0.70	0.60	0.68	0.63	0.08	0.83	<0.01	0.99
N-valerate	0.57	0.51	0.53	0.60	0.05	0.67	<0.01	0.89
Total VFA	95.53	86.95	92.42	90.30	4.70	0.72	<0.01	0.11
Acetate:propionate ratio	5.08	4.96	5.34	5.80	0.47	0.61	<0.01	0.12

[1]Control, no antibiotic growth promoters; LASA, lasalocid; SALI, salinomycin; VIRG, virginiamycin.
[2]Treat: treatment; Treat × Hour: interaction between treatment × hour.

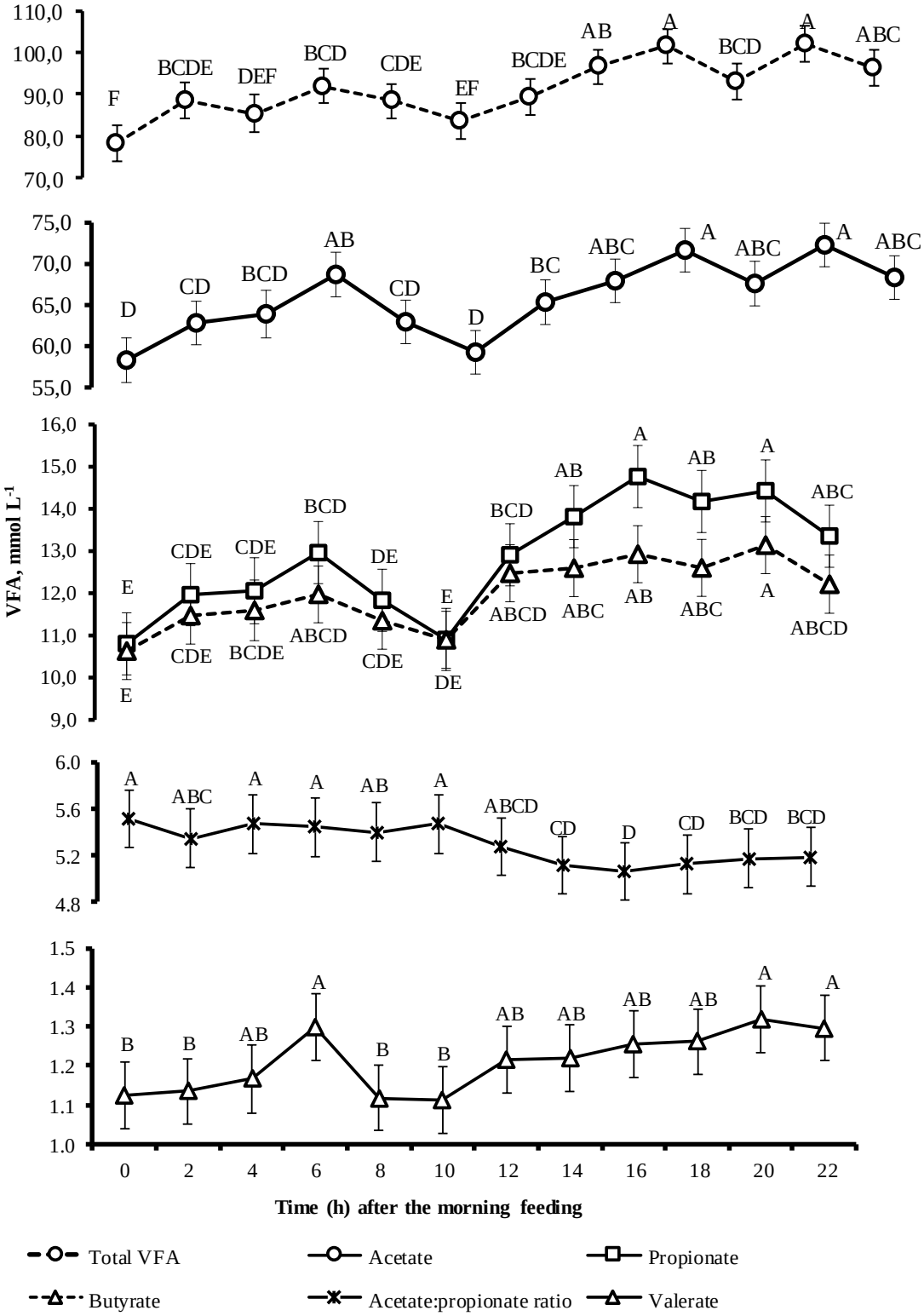

Figure 3. Effects of hour on rumen concentration of total volatile fatty acid (VFA), acetate, propionate, butyrate, acetate: propionate ratio and valerate. Arrows represent the time of diet supply. Vertical bars represent the standard deviation. Different capital letters mean that hours differ (P≤0.05).

DISCUSSION

The supply of antibiotic growth promoters did not affect the intake DM by steers. Previous studies also showed no effect of LASA (Golder and Lean, 2016), SALI (Neumann *et al*., 2016) and VIRG (Montano *et al*., 2015) on intake DM. However, others have reported that ionophores can reduce intake when used in diets with a high proportion of concentrate (Vargas *et al*., 2001). This effect may occur due to an increase in the concentration of ruminal propionic acid, which reflects an increase in energy efficiency, allowing nutritional requirements to be reached with a smaller amount of feed intake (Russel and Strobel, 1989). However, in our study, the amount of concentrate provided was not high (10g kg^{-1} of BW) and the antibiotic growth promoters did not increase the ruminal propionic acid production; thus, the absence of additive effects on intake was expected.

The lack of effects of antibiotic growth promoters on digestibility (except for digestibility of NDF) was also observed in other studies with the inclusion of ionophores (Mcallister *et al*., 1994; Neumann *et al*., 2016) or virginiamycin (Salinas-Chavira *et al*., 2009). However, ionophores can increase dietary digestibility by increasing the DM retention time in the rumen because of lower voluntary intake, stimulating rumination, and improving the ruminal environment (Ellis *et al*., 1983). However, this effect was not observed in this study because the antibiotic growth promoters did not alter intake.

Antibiotic growth promoters decreased NDF digestibility, DM and NDF disappearance in the rumen and estimation of ruminal variables. Rodrigues *et al*. (2007) observed that the use of monensin in steers fed with low quality forages, reduced the estimation of ED and potentially degradable insoluble fraction, compared to no supply of monensin. Ionophores are known to alter the microbial population of the rumen and act on cellulolytic bacteria, which can lead to a reduction in fiber degradability (Mcallister *et al*., 1994), and this may have happened our study.

The absence of effects of antibiotic growth promoters decreased on pH was also observed in other studies with the inclusion of LASA (Golder and Lean, 2016), SALI (Mcallister *et al*., 1994) or VIRG (Salinas-Chavira, 2009). However, it is accepted that ionophores increase the pH of the rumen by reducing the growth of bacteria producing lactic acid, mainly *Streptococus bovis* (Newbold and Wallace, 1988). Despite this, in our study, the additives probably did not alter ruminal pH due to the high proportion of hay in the diet (>50% of diet) inducing longer intake time, regurgitation, and saliva production, resulting in a small drop in ruminal pH and not allowing the demonstration of these antibiotics' effects. The greater pH was observed 2 h after feeding, and this occurred because the concentrate had urea in the composition, and its dissociation to ammonia in the rumen was responsible for the increase in pH.

The supply of SALI decreased the concentration of NH_3-N in the rumen. Mcallister *et al*. (1994) observed that the addition of SALI decreased the deaminase activity and ammonia production in ruminal fluid in an artificial rumen. In our study, the reduced concentration of NH_3-N caused by SALI, may be associated with the reduction in the number of bacteria that use amino acids and peptides as an energy source for their growth, and consequently, release ammonia in the ruminal environment (Yang and Russel, 1993). This reduction in the use of amino acids and peptides in the rumen favors their passage and absorption in the small intestine, increasing nitrogen efficiency (Yang and Russel, 1993). Deamination of partly ingested protein is done by bacteria that perform proteolysis, such as *Peptostreptococcus anaerobius, Clostridium aminophilum* and *Clostridim sticklandii*, all gram-positive and sensitive to the action of ionophores (Paster *et al*., 1993).

Antibiotic growth promoters decrease did not affect VFA production in the rumen. In some cases, the addition of ionophores increased the concentration of propionic acid and reduced acetic and butyric acid (Russel and Strobel, 1989; Nutrient…, 2000; Vargas *et al*., 2001; Golder and Lean, 2016). Ionophores cause alterations in the production of VFAs by modifying bacterial populations in the rumen. Gram-positive bacteria that produce acetate, butyrate, and H_2 are inhibited by ionophores, while Gram-negative bacteria, that produce propionate, have better conditions to reproduce (Bergen and Bates, 1984). However, these effects may be easier to detect in diets with high

concentrate proportions and consequently have higher production of VFA. As in our study, other studies also found no effect of ionophores (Montano *et al*., 2015; Salinas-Chavira *et al*., 2009) or virginiamycin (Montano *et al*. 2015) on the VFA production in the rumen.

CONCLUSION

Antibiotic growth promoters did not affect intake, digestibility (except for NDF), pH and volatile fatty acids in the rumen. The lasalocid tended to reduce the digestibility of NDF. The salinomycin tended to reduce the digestibility of NDF, disappearance of DM and reduced ammonia nitrogen in the rumen. The virgiamycin tended to reduce the disappearance of DM and NDF in the rumen.

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