Alexandre Arantes Miszura

Use of additives for Nellore steers in high forage diets

Pirassununga

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Doctoral thesis submitted to the Postgraduate Program in Nutrição e Produção Animal of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the PhD's degree in Sciences.

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CERTIFICADO

Certificamos que a proposta intitulada "Uso de aditivos para novilhos Nelore em dietas de alto volumoso: I. Parâmetros de fermentação ruminal, sanguíneos, digestibilidade dos nutrientes e microbiologia ruminal; II. Efeito de retirada dos aditivos sobre os parâmetros de fermentação ruminal, sanguíneos, digestibilidade dos nutrientes e microbiologia ruminal; e III. Desempenho", protocolada sob o CEUA nº 7491171017 (ID 005824), sob a responsabilidade de **Alexandre Vaz Pires** e equipe; Alexandre Arantes Miszura - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 12/12/2018.

We certify that the proposal "Use of additives for Nellore steers in high forage diets: I. Rumen fermentation parameters, blood, nutrient digestibility and ruminal microbiology; II. Effect of the withdrawal of the additives on the parameters of ruminal fermentation, blood, nutrient digestibility and ruminal microbiology; and III. Performance", utilizing 192 Bovines (192 males), protocol number CEUA 7491171017 (ID 005824), under the responsibility of **Alexandre Vaz Pires** and team; Alexandre Arantes Miszura - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 12/12/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 01/2018 a 07/2018 Área: Nutrição E Produção Animal

Origem:	Animais provenientes de outros projetos						
Espécie:	Bovinos	sexo:	Machos	Idade:	24 a 36 meses	N:	32
Linhagem:	Nelore			Peso:	350 a 400 kg		
Origem:	Animais de proprietários						
Espécie:	Bovinos	sexo:	Machos	Idade:	8 a 12 meses	N:	160
Linhagem:	Nelore			Peso:	180 a 230 kg		

Local do experimento: Laboratório de Nutrição e Produção Animal (LNRA) da Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ/USP)

São Paulo, 22 de julho de 2019

Profa. Dra. Anneliese de Souza Traldi
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretária
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

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Doctoral thesis submitted to the Postgraduate Program in Nutrição e Produção Animal of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the PhD's degree in Sciences.

Date: _____/_____/_____

Committee Members

Prof. _____

Institution: _____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

To my parents, Jorge Miszura and Claudete Arantes Lourenço Miszura, for their unconditional efforts, support, and love.

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To God, which I have faith in and wouldn't come here without him.

“Facts are stubborn things; and whatever may be our wishes, our inclinations, or the dictates of our passions, they cannot alter the state of facts and evidence.”

John Adams

RESUMO

Miszura, A. A. **Uso de aditivos para novilhos Nelore em dietas de alto volumoso**. 2021. 77 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2021.

Este estudo teve como objetivo comparar os efeitos dos aditivos alimentares lasalocida, narasina e virginiamicina para bovinos alimentados com dietas de alta forragem. A hipótese foi de que a inclusão desses aditivos melhoraria a digestibilidade dos nutrientes, alteraria a fermentação ruminal e melhoraria o desempenho de bovinos de corte alimentados com dietas de alta forragem. Foram delineados dois experimentos para avaliar os efeitos das moléculas lasalocida, narasina e virginiamicina sobre a digestibilidade aparente do trato total, parâmetros de fermentação ruminal, sanguíneos e desempenho de bovinos Nelore (*Bos indicus*) alimentados com dieta de alto volumoso. No experimento 1, 32 novilhos Nelore providos de cânula ruminal (peso corporal em jejum = 355 ± 4,4 kg) foram aleatorizados em blocos (n = 8), de acordo com seu peso corporal em jejum. Os novilhos foram alimentados diariamente e as dietas eram compostas de 99% de pré-secado de Coastcross e 1% de concentrado, usado como veículo de fornecimento dos aditivos. As dietas experimentais consistiam em 1) dieta baseada em forragem sem aditivos (CON); 2) 13 mg/kg de MS de narasina (NAR); 3) 20 mg/kg de MS de lasalocida e 4) 20 mg/kg de MS de virginiamicina (VM). O período experimental durou 140 dias, divididos em 5 períodos de 28 dias cada. A inclusão dos aditivos não afetou o CMS ($P = 0,46$), consequentemente, não afetou o consumo de nutrientes ($P > 0,05$). Além disso, não houve diferença na digestibilidade dos nutrientes entre as dietas ($P > 0,05$). Houve uma interação tratamento x dia ($P < 0,01$) para AGCC, acetato, propionato e a taxa acetato:propionato (A:P). Os animais que consumiram NAR tiveram os maiores valores de AGCC nos dias 84 e 112 ($P < 0,05$), enquanto as concentrações de acetato foram as menores para NAR nos dias 28, 56, 112 ($P < 0,05$), e menor no dia 140 em relação ao CON e LAS ($P < 0,05$). O tratamento NAR teve o maior valor de propionato e menor A:P nos dias 28, 56, 112 e 140 quando comparado com os outros tratamentos ($P < 0,05$). No experimento 2, 160 novilhos Nelore foram blocados (n = 10) pelo peso inicial em jejum (212 ± 3,1 kg) em um confinamento de 140 dias de duração. Os novilhos foram alimentados diariamente e as dietas foram compostas de 96% de pré-secado de Coastcross e 4% de concentrado, usados como veículo de fornecimento para os aditivos. O

tratamento NAR teve o maior GMD (P = 0,04) do que CON e VM e foi similar para LAS. Por outro lado, os animais que receberam LAS apresentaram um GMD similar a estes outros tratamentos. A eficiência alimentar (GMD/CMS) foi maior (P = 0,05) para NAR em comparação ao grupo CON e VM. Além disso, o fornecimento de LAS aumentou (P = 0,05) a eficiência alimentar comparado ao CON, com nenhuma diferença comparado a VM. Consequentemente, o tratamento NAR teve o maior peso final (P = 0,03) do que os outros tratamentos. Em conclusão, NAR melhorou os parâmetros e desempenho de novilhos alimentados com dietas de alta forragem e LAS melhorou a eficiência alimentar.

Palavras-chave: Aditivos alimentares, lasalocida, narasina, virginiamicina, parâmetros ruminais

ABSTRACT

Miszura, A. A. **Use of additives for Nellore steers in high forage diets**. 2021. 77 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2021.

This study aimed to compare the effects of the feed additives lasalocid, narasin and virginiamycin to cattle fed high-forage diet. The hypothesis was that the inclusion of these additives would improve nutrient digestibility, change rumen fermentation and, increase the performance of beef cattle fed high-forage diet. Two experiments were designed to evaluate the effects of lasalocid, narasin, and virginiamycin on apparent total tract nutrient digestibility, rumen fermentation characteristics, blood parameters, and performance of Bos indicus cattle fed high-forage diet. In Exp. 1, 32 rumen-fistulated Nellore steers (initial shrunk BW = 355 ± 4.4 kg) were assigned to a randomized complete block design (n = 8), according to their initial shrunk BW. Steers were fed daily and diets were composed of 99% of coastcross haylage and 1% of concentrate, used as a delivery vehicle for the additives. The experimental diets consisted of 1) forage-based diet without feed additives (CON); 2) 13 mg/kg DM of narasin (NAR); 3) 20 mg/kg DM of lasalocid (LAS); and 4) 20 mg/kg DM of virginiamycin (VM). The experimental period lasted 140 d, divided into 5 periods of 28 d each. The inclusion of feed additives did not affect the DMI ($P = 0.46$), consequently, did not affect the nutrients intake ($P > 0.05$). In addition, there was no difference in nutrient digestibility among diets ($P > 0.05$). There was an interaction between treatment x day ($P < 0.01$) for total SCFA, acetate, propionate, and acetate:propionate (A:P) ratio. Animals offered NAR had the greatest total SCFA values on days 84 and 112 ($P < 0.05$), whereas acetate concentration was lowest to NAR on days 28, 56, 112 ($P < 0.05$), and lower on day 140 in relation to CON and LAS($P < 0.05$). The treatment NAR had the greatest propionate and lowest A:P ratio values on days 28, 56, 112 and 140 when compared to other treatments($P < 0.05$). In Exp. 2, 160 Nellore yearling bulls were blocked (n = 10) by initial shrunk BW (212 ± 3.1 kg) in a 140-d feedlot trial. Bulls were fed daily and diets were composed of 96% of coastcross haylage and 4% of concentrate, used as a delivery vehicle for the additives. The treatment NAR had a greater ADG ($P = 0.04$) than CON and VM and was similar to the LAS. In turn, the animals that received LAS presented a similar ADG to those of the other treatments. The gain:feed (G:F) was greater ($P = 0.05$) for NAR than CON and VM. Besides, LAS

supplementation increased (P = 0.05) the G:F compared with CON, with no difference compared to VM. Consequently, the treatment NAR had the greatest final BW (P = 0.03) than others. In conclusion, NAR improves rumen parameters and performance of yearling bulls fed high-forage diets and LAS improves feed efficiency.

Keywords: Feed additives, lasalocid, narasin, ruminal parameters, virginiamycin

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The manuscript was written in compliance to guidelines of the *Journal of Animal Science*.

REVIEW OF THE LITERATURE

1. INTRODUCTION

In Brazil, the cattle herd are about 215 million heads, in an area of 162 million hectares. This corresponds to a stocking rate of approximately 0.93 UA/ha. In 2020, 43.3 million heads were slaughtered, which corresponds to an outcome rate of 20.9%. Of this slaughter volume, about 86% are grazing animals (ABIEC, 2020), a predominant feature of the Brazilian beef production system.

One of the advantages in the production of animals in pastures is due to its low cost. However, the low performance indexes of Brazilian livestock are obstacles to a more efficient production. As one of the challenges to maximize the activity of beef cattle in Brazil, can mention the seasonality in the production of forage. In addition, diets containing large amounts of forage usually lead to a high rate of acetate, and low propionate in rumen fermentation (Van Soest, 1994), being unfavorable from the energy efficiency in rumen fermentation.

The tropical grasses, although they have high production, have poor quality when compared to temperate grasses. And often tropical grasses do not meet the mineral requirements of animals produced in this type of system, and supplementation is required for better performance (McDowell and Arthington, 2005). In this case, mineral supplementation becomes indispensable, and mineral supplementation increases animal performance by correcting some mineral deficiency of the animal (Fieser et al., 2007).

Feed additives are one of the tools that can improve the conversion of forage into animal protein and increase nutrient utilization efficiency by reducing losses from fermentative routes leading to methane and carbon gas production (Tedeschi et al., 2003). Based on the actions of ionophores to increase propionate and reduce rumen ammonia,

ruminants could respond to ionophore supplementation when fed diets containing large amounts of forage (Beede et al., 1986).

According to normative 15/2009 (MAPA), additive is a substance, microorganism or formulated product, intentionally added to products, which is not used as an ingredient, whether or not it has nutritional value and which improves the characteristics of local products to animal feed or of animal products, improve the performance of healthy animals and serve as nutritional needs or have an anticoccidial effect.

The use of additives in mineral mixtures is an economical alternative and may well be accepted by producers as it does not generate changes in the routine of activities on the farm. However, the literature is quite divergent regarding the effect of additive use on grazing animals. This may be explained by the daily variation in mineral supplement intake. Another problem encountered with the addition of ionophores to mineral mixtures is the reduction in supplement intake, which results in lower additive consumption and lower animal performance (Bagley et al. 1988).

Despite the numerous benefits of using additives in cattle nutrition, most research focuses on high concentrate diets (Tedeschi et al., 2003; Duffield et al., 2012; Ellis et al., 2012). Also, most data in the literature on the use of these additives refer to specific groups, such as monensin (Duffield et al., 2012; Ellis et al., 2012), a molecule that has difficulty in supply via mineral mix due to decreased intake by animals (Beck et al., 2014; Fieser et al., 2007). One of the reasons that question the efficiency of supplementary ionophore supply is the frequency of consumption exerted by the animal, leaving the question of whether the animal is consuming the necessary amount of supplement and additive. Since mineral supplement intake also varies among animals (Goulart, 2010).

In this sense, there is an important gap to be filled by research involving the use of molecules capable of altering the fermentation process in ruminants fed with high roughage diets.

1.1 Narasin

Definition. The narasin is a polyether antibiotic (Figure 1) ionophore produced by *Streptomyces aureofaciens* with empirical formula $C_{43}H_{72}O_{11}$ and molecular weight of 764 g/mol (Berg and Hamill, 1977). The narasin is active *in vitro* against Gram-positive bacteria, anaerobic bacteria and, fungi. The cation selectivity is $Na^+ > K^+$, $Rb^+ > Cs^+ > Li^+$, being soluble in alcohol, acetone, chloroform, and ethyl acetate. The cation selectivity of polyether ionophores is largely due to the size of the cavity which has the oxygen atoms as ligands facing inside. Thus, the cavity provided by narasin can best accommodate the Na^+ ion (Wong et al., 1977).

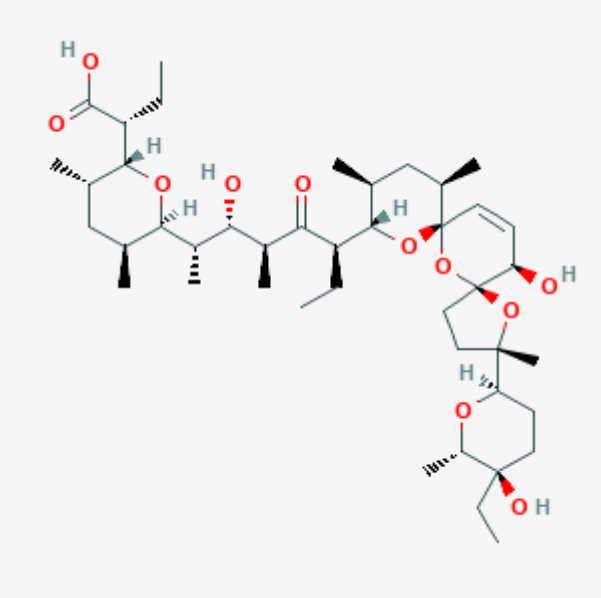

Figure 1. Narasin chemical structure (PubChem, 2021)

The narasin and salinomycin are similar in chemical structure except that narasin has an additional methyl group on the 6-member cyclic ether at the carboxylic terminal. Like other polyether monocarboxylic ionophores, monensin, the narasin inhibited the mitochondrial ATPase induced by active transport of alkali metal cations (Wong et al., 1977).

Mechanism of action. The narasin is well known by poultry like coccidia control (Jeffers et al., 1988) and growth promoter to finishing swine (Arkfeld et al., 2015). Interesting, narasin administration to finishing pigs resulted in an improved apparent nitrogen digestibility, decreasing fecal nitrogen, and increasing the relative proportion of propionic acid in the large intestine (Wuethrich et al., 1998).

The ionophores fed to cattle (monensin, lasalocid, salinomycin, and narasin) are describe in the literature like the similar mechanism of action. As described by Russell and Houlihan (2003), most of susceptible microorganisms maintain a higher concentration of potassium inside than outside the cells, normally expelling sodium and protons. The Gram-positive bacteria affected by monensin (Figure 2), occur a rapid efflux of potassium and an influx of sodium and protons. The cells respond by activating membrane ATPases and transporters, when they end up spending their energies.

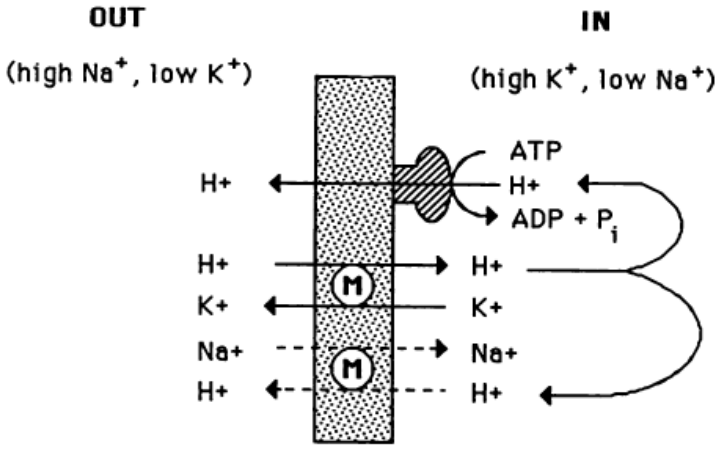

Figure 2. Mechanism of action by ionophores (adapted of Russell and Houlihan, 2003).

There is few information in the literature to narasin administration to ruminants compared to monensin. In a study evaluating the effects of antimicrobials compounds on *in vitro* fermentation using ruminal fluid, the narasin and salinomycin were more inhibitory than lasalocid and monensin on lactic acid production. Besides that, narasin and salinomycin were the most effective in enhancing propionate production and reducing acetate:propionate ratio than lasalocid and monensin (Nagaraja et al., 1987). According to the authors, the optimum dosage of narasin and salinomycin is about threefold less than that of lasalocid and monensin.

Rumen fermentation. Regarding ruminal fermentation parameters, Polizel et al. (2016a) evaluated the effects of providing increasing concentrations of narasin (0, 8, 16, 24 and 32 mg/kg DM) for lambs fed high forage diets. The authors found that the total concentration of SCFA increased linearly with the inclusion of narasin doses. Also, the inclusion of narasin doses did not affect DMI.

In another study, Polizel et al. (2018a) evaluated the ruminal fermentation parameters of cattle fed high forage diets receiving 0 mg narasin/kg DM (control), 13 mg narasin/kg DM and 20 mg narasin/kg DM. They observed that narasin doses increased the molar proportion of propionate, decreased the molar ratio of acetate, and consequently altered the ratio of acetate: propionate. These changes in ruminal fermentation parameters were obtained when the supply of narasin was performed once a day, every 24 hours.

In general, on metabolism trials in high forage diets, the narasin increase total SCFA (14.3%), propionate (9.5%), and decrease acetate (1.6%) and acetate:propionate ratio (10.8%), when compared to control (Tabela 1).

Table 1. Summary of rumen fermentation trials with narasin, published like an abstract in the annual meeting of ASAS

Author	Specie	Category	N[1]		Diet type[2]	CP[3]	NDF[4]	DF, d[5]	Dose, mg/kg DM	Response %[6]					
			Com	Nar						Total SCFA	Acetate	Propionate	Ac:Prop	Amonia	pH
Polizel et al. (2016b)	sheep	wethers	5	5	High-forage	6.8	67.2	20	8, 16, 24, and 32	+14.21	NS	NS	NS	NS	NS
Polizel et al. (2018a)	cattle	steers	10	10	High-forage	8.6	68	140	13 and 20	+10.82	-1.75	+12.57	-13.2	-	NS
Miszura et al. (2018)	cattle	steers	8	8	High-forage	11.6	64.6	140	13	+14.98	-2.36	+10.74	-12.74	NS	NS
Oliveira et al. (2018)	sheep	lambs	4	4	High-forage	16.2	71.4	36	13, 26, and 39	+17.19	NS	+8.47	-9.87	NS	NS
Limede et al. (2019a)	cattle	steers	8	8	High-forage	20.70	58.9	140	13	NS	-0.68	+6.16	-7.42	NS	NS
Means										**+14.3**	**-1.6**	**+9.5**	**-10.8**	**-**	**-**

[1]N: experimental unit.
[2]Diet type: high forage defined like more than 50% of the diet.
[3]CP: crud protein of forage.
[4]NDF: Neutral detergent fiber of forage.
[5]DF: days of feeding.
[6]Response: positive (+) means increased effect compared to control; negative (-) means decreased effect compared to control; NS means non-significant effect.

Performance. In performance trials, a comparison of monensin, narasin, salinomycin, and tylosin on feedlot performance of steers found that narasin plus tylosin improved average daily gain on a carcass basis by 7.4% and improved feed conversion by 5.3% over monensin alone (Strasia et al., 1987). The dose used was about 11 mg/kg DM.

In most recent studies, Silva et al. (2015) evaluated the inclusion of narasin in the mineral mixture at concentrations of N0 (control), N6.5 (650 mg/kg MM) and N13 (1300 mg/kg MM) for Nellore heifers fed roughage-based diets. They concluded that the animals that received the N13 treatment presented higher ADG (582g/day) when compared to the control treatment (489g/day). Also, DMI and mineral mix consumption was not affected by experimental diets.

Evaluating supplement intake by grazing beef bulls, Cappellozza et al. (2019) did not found reduce supplement intake by narasin inclusion. Besides that, the authors found an increased the frequency of visits to the feeder for mineral salt containing narasin. In this trial, the supplements were formulated to deliver 120 to 150 mg of narasin per head/day.

To evaluate the effect of narasin on lamb performance, Polizel et al. (2016c) used increasing concentrations of narasin (5, 10 and 15mg/kg DM), control (without ionophore) and 25 mg monensin/kg DM in diets containing high concentrate. The inclusion of narasin linearly increased the final weight and feed efficiency of the animals, just as narasin fed animals had higher final weight when compared to monensin treated animals.

In a study by Polizel et al. (2017) the authors evaluated the supply of 0, 71.5 and 110 mg of narasin/day through the mineral mixture to newly weaned calves grazing. The

authors reported that the inclusion of narasin did not affect the mineral intake. In addition, narasin-fed animals showed an increase in ADG of 16.63% (dose 71.5 mg narasin/day) and 18.66% (dose 110 mg narasin/day) compared to the control treatment.

In a comparative study, Limede et al. (2019b) evaluate the effects of narasin, salinomycin, and flavomycin on performance of beef cattle consuming a high-forage diet (96%). There was no effect on feed efficiency but, the narasin inclusion increased DMI, ADG, and final BW in comparison with the others and group control.

In general, on performance trials in high forage diets, the narasin increased DMI (7.4%), ADG (15.2%), and FE (16.7%) when compared to control (Table 2). In Brazil, narasin is allowed to beef cattle since 2015 in dosage of 5 - 13 mg/kg of DMI (5 to 13 ppm), equivalent to 0.10 to 0.26 mg of narasin / kg of BW of the animals.

Table 2. Summary of performance trials with narasin, published like an abstract in the annual meeting of ASAS

Author	Specie	Category	N[1]		Diet type[2]	CP[3]	NDF[4]	DF, d[5]	Dose, mg/kg	Entry BW, kg	Response %[6]		
			Control	Narasin					DM		DMI	ADG	FE
Silva et al. (2015)	cattle	heifers	10	20	High-forage	11.8	73.4	28	6.5 and 13	222	NS	+16.49	+17.66
Miszura et al. (2019)	cattle	Bulls	10	10	High-forage	12.0	62.0	140	13	212	+7.55	+19.03	+15.78
Limede et al. (2019b)	cattle	Bulls	10	10	High-forage	19.30	61.7	140	13	298	+7.18	+12.67	NS
Polizel et al. (2017)	cattle	Bulls	10	20	Grazing	13.7	68.5	84	13 and 20	177	-	+14.26	-
Polizel et al. (2018b)	cattle	Bulls	16	16	Grazing	13.5	67.8	112	13	193	-	+13.14	-
Polizel et al. (2019)	cattle	Bulls	15	15	Grazing	12.1	71.1	112	13	322	-	+15.49	-
Mean						**13.7**	**67.4**	**102.7**		**237.3**	**+7.4**	**+15.2**	**+16.7**

[1]N: experimental unit.
[2]Diet type: high forage defined like more than 50% of the diet.
[3]CP: crude protein of forage.
[4]NDF: Neutral detergent fiber of forage.
[5]DF: days of feeding.
[6]Response: positive (+) means increased effect compared to control; negative (-) means decreased effect compared to control; NS means non-significant effect.

1.2 Lasalocid

Definition. The lasalocid was the first ionophore to be identified in 1951, when X-537A (later called lasalocid) was isolated from a bacterium *Streptomyces sp.* (Berger et al., 1951). The lasalocid (Figure 3) is an ionophore antibiotic produced by *Streptomyces lasaliencis* with molecular formula $C_{34}H_{53}O_8Na$ and a molecular weight of 612.78 with cation selectivity for Ba^{2+}, $K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$ (Nagaraja, 1997).

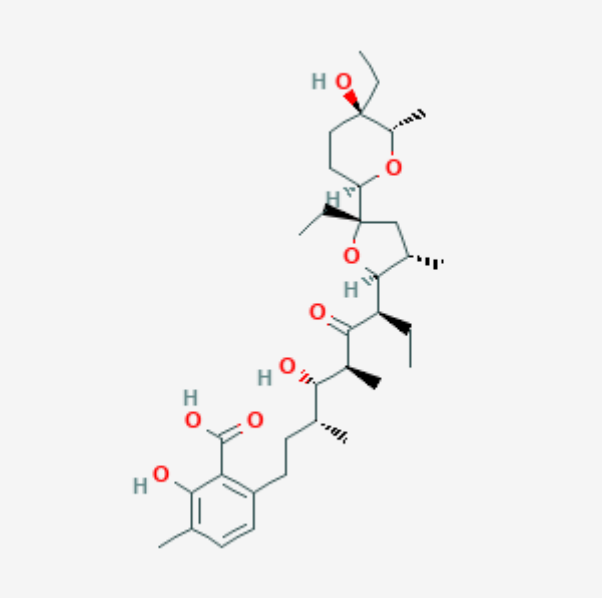

Figura 3. Lasalocid sodium structural formula (PubChem, 2021)

Mechanism of action. This ionophore can translocate mono and divalent cations across the bacterial cell membrane (Bergen & Bates, 1984), resulting in a modified rumen environment through the death or impaired growth of mainly Gram-positive ruminal bacteria. The mechanism of action is described like the same of the others ionophores, specifically monensin, like described in Narasin section.

Rumen fermentation. Part of the ionophore effect is a result of decreased methane and increased propionate in rumen fermentation (Bergen & Bates, 1984; Russell & Strobel, 1989). Jacques et al. (1987) found no differences for lasalocid compared to the control group in rumen pH, N ammonia, total VFA concentration, molar proportion of VFA, or ruminal fluid flow measurements.

In a recent review by Golder and Lean (2016) on the use of lasalocid in both high forage and high concentrate diets, analyzing 10 studies on rumen fermentation (only two in pasture), there was an increase in total concentrations of VFA and rumen ammonia. The lasalocid increased the molar percentage of propionate and decreased acetate and butyrate (4.62, 3.18 and 0.83% respectively) compared to the control.

In the metabolism studies evaluated, the mean dose used was 240 ± 126 mg/day and 70 ± 44 days of delivery. The meta-analysis of ruminal fermentation measurements mainly of beef cattle, although not of the same magnitude as performance studies, showed findings consistent with known ionophore mechanisms, which increase the molar proportion of propionate and decrease the molar proportion of acetate and butyrate.

In general, metabolism data when not discriminated by diet type (Golder and Lean, 2016) suggest that the lasalocid has effects on ruminal parameters like monensin. Thus, an increase in propionate molar ratio, a decrease in acetate production and an improvement in rumen energy efficiency is expected with a decrease in methane production. However, there is little consistent data regarding the high forage diet. In Golder and Lean (2016) meta-analysis, ruminal fermentation data for lasalocid in grazing animals were inconsistent, requiring further studies.

Performance. Concerning performance, the lasalocid has shown the potential to improve weight gain and feed efficiency of ruminants (Bergen & Bates, 1984). Page (2003) presented data from 15 studies on the supply of lasalocid to grazing beef cattle, where the animals were supplemented with concentrate and the experiments lasted between 84 and 112 days. The maximum performance for grazing animals occurred with lasalocid doses between 200 and 300 mg/head/day (Table 3).

Tabela 3. The maximum performance for grazing animals regarding lasalocid doses in mg/animal/day

Dose (mg/animal/day)	ADG (kg/day)	Compared to control (%)
0	0.57	-
50	0.58	1.75
100	0.6	5.26
200	0.64	12.28
300	0.65	14.04

Adapted de Page (2003).

Bretschneider et al. (2008) reviewed 48 scientific articles on the use of growth-promoting antibiotics in grazing cattle feed, including lasalocid. The average dose used for lasalocid was 68 ± 19 mg / 100 kg body weight. In general, the ionophore was able to increase by 10.3% the average daily gain compared to control treatments. According to the authors, the animals responded quadratically to lasalocid doses (Figure 4), with maximum responses at doses ranging from 80 to 100 mg of ionophore per 100 kg body weight.

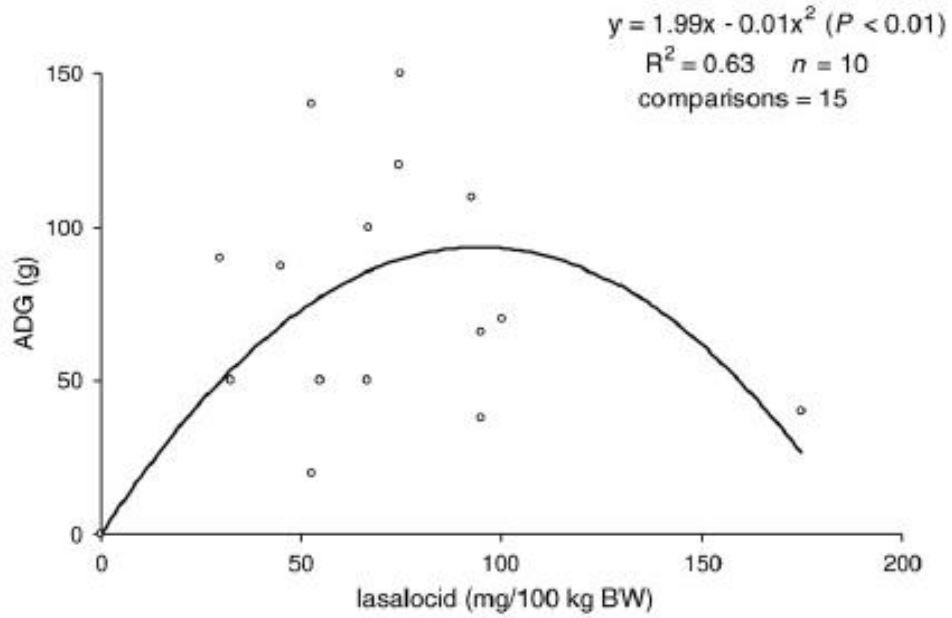

Figura 4. The average daily gain according lasalocid dose (adapted from Bretschneider et al., 2008)

Regarding forage intake, there was no effect of lasalocid use regardless of the dose used (Figure 5). Due to the positive effect of ionophores on the weight gain of animals without a change in forage intake, it was observed that the animals responded quadratically regarding feed conversion, with maximum values at doses between 80 and 100 mg / 100 kg body weight.

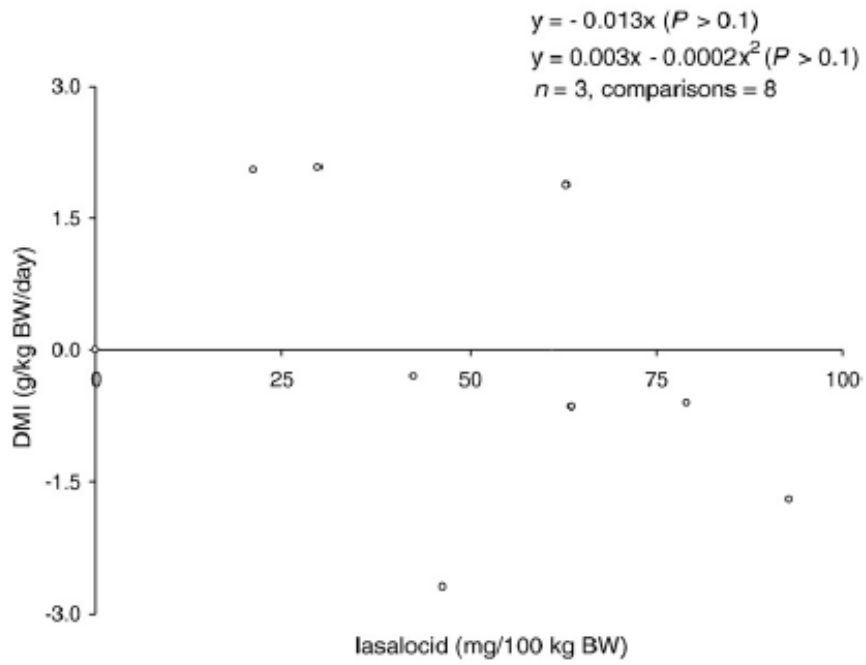

Figura 5. The effect of lasalocid on the dry matter intake (Adapted from Bretschneider et al., 2008)

In the Golder and Lean (2016) meta-analysis, 31 studies with beef cattle performance were evaluated, where ADG increased about 40g/day and improved feed efficiency without affecting DMI. In performance experiments, the average dose used was 232 ± 108 mg/day for 126 ± 105 days.

The performance data presented, which have many works with high forage fed animals, confirm its potential as a performance enhancer with increasing ADG, not affecting the consumption of DM, thus increasing the feed efficiency. In Brazil, the lasalocid dose allowed is between 100-340mg/head/day for animals growing (Ministério da Agricultura, Pecuária e Abastecimento).

2.3 Virginiamycin

Definition. The virginiamycin (Figure 6) is an antibiotic non-ionophore produced by *Streptomyces virginiae* with molecular formula $C_{28}H_{35}N_3O_7$ (factor M1) and $C_{43}H_{49}N_7O_{10}$ (factor S1), and molecular weight of 1349.5 g/mol (Cocito, 1979).

In general, virginiamycin is active against Gram-positive bacteria, not acting against Gram-negative, as it cannot penetrate the bacterial outer membrane. Eukaryotes, fungi, yeast, algae, and plants are not sensitive to the molecule. *In vitro* studies indicate that virginiamycin acts on protein metabolism by preventing its degradation (Dierick et al., 1980).

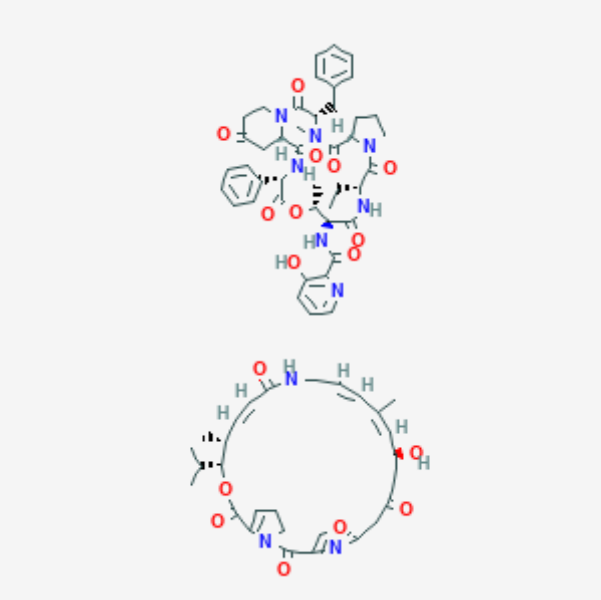

Figura 6. Virginiamycin S1 and M1 structural formula (PubChem, 2021).

Mechanism of action. At the intracellular level, the factors M and S bind specifically and irreversibly to 50S ribosomal subunits (Figure 7), inhibiting the formation of peptide binding during protein synthesis. It penetrates through the cell wall of Gram-positive bacteria, binding to ribosome subunits in the cytoplasm, thereby inhibiting the formation of peptide bonds during protein synthesis (Cocito 1979; Cocito and Chinali 1985; Di Giambattista et al. 1989). Metabolic processes are disrupted in the microorganism,

resulting in inhibition of multiplication and eventually cell death. After inhibition of protein synthesis, the effect of virginiamycin remains the same after its withdrawal (short one) in culture medium for 30 minutes (Cocito, 1979).

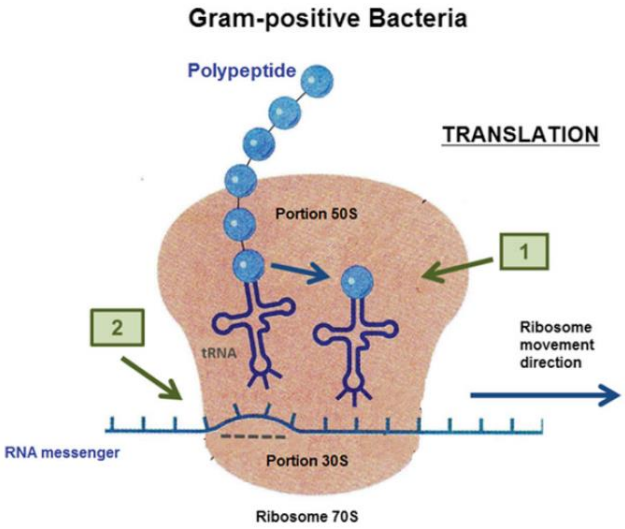

Figura 7. Mechanism of action of virginiamycin (adptaded from De Araujo et al., 2016)

Rumen fermentation. The microorganisms that produce lactic acid, butyric acid, formic acid, and hydrogen are susceptible to virginiamycin, and bacteria that produce succinic acid or ferment lactic acid are resistant. Another important inhibited microorganism is *Fusobacterium necrophorum*, an important etiological agent of liver abscess in cattle. In high energy diets, the main effect is on rumen lactic acid concentration. It is active against *Streptococcus bovis* and *Lactobacillus ruminis*, preventing the development of dangerous rumen lactic acid levels (De Araújo et al., 2016).

Performance trials. When fed to grazing animals, virginiamycin has been shown to increase the weight gain rate and improves feed efficiency. Virginiamycin is considered to improve feed efficiency and growth rate in cattle by modulating the rumen environment, potentially improving digestion and nutrient absorption (De Araújo et al., 2016).

In an experiment evaluating the virginiamycin inclusion on mineral mixture to grazing beef cattle, Goulart (2010) did find a 10% ADG increase to virginiamycin compared to control. In this study, the dose consumed was 98 mg/head/day. Besides that, there was a tendency to virginiamycin decrease the supplement intake, an unexpected effect of this additive according to the literature.

Searching for the best dose to grazing beef cattle, Alves Neto (2014) evaluated 0, 35, 55, and 75 mg of virginiamycin on mineral supplement. The metabolism trial did not find differences but, the author did find a quadratic response in average daily gain, with the dose of 46.75 mg/100 kg of BW being the best, according the equation.

In another study with virginiamycin on mineral mixture to grazing animals, there was a 13.8% increase on ADG compared to control (Costa, 2016). In this trial, virginiamycin did not affect the intake of mineral supplement (40 mg/100 kg of BW).

The recent analysis of the effectiveness of virginiamycin on liver abscess incidence and growth performance in feedlot cattle, Tedeschi and Gorocica-Buenfil (2018) indicated that virginiamycin increased ADG about 2.3 times than monensin for the same dosage and feeding period length. Also, the authors conclude that virginiamycin is effective in reducing liver abscesses incidence when fed between approximately 12 and 24 mg/kg of DM, and the maximum reduction might occur at approximately 24 mg/kg of DM.

In Brazil, according to Phibro Animal Health, the dose of virginiamycin ranges from 100 mg to 340 mg/animal/day.

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EFFECTS OF LASALOCID, NARASIN, AND VIRGINIAMYCIN ON INTAKE, DIGESTION, RUMEN FERMENTATION CHARACTERISTICS AND PERFORMANCE OF *BOS INDICUS* CATTLE FED A HIGH-FORAGE DIET

ABSTRACT

Two experiments were designed to evaluate the effects of lasalocid, narasin, and virginiamycin on apparent total tract nutrient digestibility, rumen fermentation characteristics, blood parameters, and performance of *Bos indicus* cattle fed high-forage diet. In Exp. 1, 32 rumen-fistulated Nellore steers (initial shrunk BW = 355 ± 4.4 kg) were assigned to a randomized complete block design (n = 8), according to their initial shrunk BW. Steers were fed daily and diets were composed of 99% of coastcross haylage and 1% of concentrate, used as a delivery vehicle for the additives. The experimental diets consisted of 1) forage-based diet without feed additives (**CON**); 2) 13 mg/kg DM of narasin (**NAR**); 3) 20 mg/kg DM of lasalocid (**LAS**); and 4) 20 mg/kg DM of virginiamycin (**VM**). The experimental period lasted 140 d, divided into 5 periods of 28 d each. The inclusion of feed additives did not affect the DMI ($P = 0.46$), consequently, did not affect the nutrients intake ($P > 0.05$). In addition, there was no difference in nutrient digestibility among diets ($P > 0.05$). There was an interaction between treatment x day ($P < 0.01$) for total SCFA, acetate, propionate, and acetate:propionate (**A:P**) ratio. Animals offered NAR had the greatest total SCFA values on days 84 and 112 ($P < 0.05$), whereas acetate concentration was lowest to NAR on days 28, 56, 112 ($P < 0.05$), and lower on day 140 in relation to CON and LAS ($P < 0.05$). The treatment NAR had the greatest propionate and lowest A:P ratio values on days 28, 56, 112 and 140 when compared to other treatments ($P < 0.05$). In Exp. 2, 160 Nellore yearling bulls were blocked (n = 10) by initial shrunk BW (212 ± 3.1 kg) in a 140-d feedlot trial. Bulls were fed daily and diets were composed of 96% of coastcross haylage and 4% of concentrate, used as a delivery vehicle for the additives. The treatment NAR had a greater ADG ($P =$

0.04) than CON and VM and was similar to the LAS. In turn, the animals that received LAS presented a similar ADG to those of the other treatments. The gain:feed (**G:F**) was greater (P = 0.05) for NAR than CON and VM. Besides, LAS supplementation increased (P = 0.05) the G:F compared with CON, with no difference compared to VM. Consequently, the treatment NAR had the greatest final BW (P = 0.03) than others. In conclusion, NAR improves rumen parameters and performance of yearling bulls fed high-forage diets and LAS improves feed efficiency.

Key words: *Bos indicus*, feed additives, forage, performance, propionate

INTRODUCTION

In general, beef production systems required about 81% of forage during cattle production cycle (Watson et al., 2015). The feed additives are important tools that can promote better nutrient utilization by reducing losses associated with the fermentative routes that lead to the production of methane and carbon dioxide (Tedeschi et al., 2003). Most of the research conducted to date within feed additives focused on high-concentrate diets (Tedeschi et al., 2003; Duffield et al., 2012; Ellis et al., 2012), and most of which are monensin studies (Duffield et al., 2012; Ellis et al., 2012). Hence, there is an important gap to be filled by research involving the use of molecules capable of altering the fermentation process in ruminants fed diets containing high-forage levels (Fieser et al., 2007; Beck et al., 2014). Lasalocid is an important ionophore in beef cattle production, that can improve propionate production and performance of beef cattle fed high-forage diets (Golder and Lean, 2016). Narasin is an ionophore which improves performance of grazing beef cattle (Silva et al., 2015; Polizel et al., 2018) and does not impacts mineral supplement intake (Cappellozza et al., 2018; Polizel et al., 2018). Another option is the use of virginiamycin, a non-ionophore antibiotic that positively impact ruminal fermentation and performance of grazing beef cattle (Araújo et al., 2016). This study aimed to compare the effects of these feed additives to cattle fed high-forage diet. We hypothesized that the inclusion of lasalocid, narasin and virginiamycin would improve nutrient digestibility, change rumen fermentation and, increase the performance of beef cattle fed high-forage diet.

MATERIALS AND METHODS

Experimental procedures involving animals were reviewed and approved by the Ethics Committee on Use of Animals of School of Veterinary Medicine and Animal Science (University of Sao Paulo; CEUA/FMVZ; protocol number 7491171017).

EXPERIMENT 1

Animals, housing, and Experimental design

Thirty-two rumen-fistulated Nellore (*Bos indicus*) steers were assigned to a randomized complete block design (n = 8), according to their initial shrunk body weight (**SBW =** 355 ± 4.4 kg and age = 24 ± 1.0 mo). The experimental period lasted 140 d and was divided into 5 periods of 28 d each (0, 28, 56, 84, 112 and 140d). The steers were kept indoors, in an individual tie-stall system (6 m^2), with a concrete floor, feed bunk, and waterer.

Feeding Management and Treatments

Steers were fed daily and diets were composed of 99% of chopped coastcross haylage [Cynodon dactylon (L.) Pers] and 1% of concentrate (50% ground corn and 50% ground soybean hulls), used as the delivery vehicle for the additives (Table 1). The coastcross haylage was chopped using a vertical mixer (Mixer VM8B, DeLaval International AB, Tumba, Sweden) for about 15 min (Final particle length of: 53.3 ± 3.5% > 19 mm; 21.9 ± 1.7% > 8 mm; 12.4 ± 1.0% > 4 mm; and 12.4 ± 1.5% on bottom sieve). Steers were randomly assigned to receive the concentrate in addition to 1 of 4 feed additives treatments: 1) 13 mg/kg DM of narasin (**NAR**; Zimprova, Elanco Animal Health, Sao Paulo, SP, Brazil), 2) 20 mg/kg DM of sodium lasalocid (**LAS**; Taurotec, Zoetis, Sao Paulo, SP, Brazil), 3) 20 mg/kg DM of virginiamycin (**VM**; V-Max, Phibro Animal Health Corporation, Guarulhos, SP, Brazil), and 4) concentrate with no feed additives added (**CON)**. The feed additives were mixed into the concentrate and offered to each steer individually. Steers promptly consumed additives and concentrate within 20 min after feeding and then the haylage was offered.

Sample collection, laboratory Analyses, and Measurements

The feed and orts were recorded daily to determine the DMI. Samples of haylage and concentrate were collected weekly, pooled across all weeks within each period, and analyzed for the nutrient profile. From day 23 to 27 of each experimental period, total fecal production was individually collected to determine nutrient digestibility in the total digestive tract. Total fecal production was quantified using an electronic scale (Marte AC-10K; Marte Cientifica, Sao Paulo, SP, Brazil) at 0800 h and 1600 h, and a representative sample (10%) of the daily production of each steer was collected and stored at -18°C on the same day of collection.

Samples of feed, orts, and feces were dried in a forced-air oven at 60°C (AOAC, 1990; #930.15). Sequentially, the samples were ground through a 1-mm Wiley Mill screen (Marconi, Piracicaba, SP, Brazil). The final DM content was determined after oven-drying the samples at 105°C (AOAC, 1990; #934.01) and ash concentration was obtained by incinerating the samples in an oven at 550°C for 4 h (AOAC, 1990; method #942.05). Sequential detergent fiber analyses were used to determine NDF (Van Soest et al., 1991) and ADF (Goering and Van Soest, 1970) with an Ankom 2000 fiber analyzer (Ankom Tech. Corp., Macedon, NY, USA). Sodium sulfite and heat-stable α-amylase were added in the NDF analysis. The total N was determined according to AOAC (1990; method #968.0) using the Leco TruMac N (Leco Corp., St. Joseph, MI, USA) and the crude protein (CP) was obtained by multiplying the total N content by 6.25. Determination of ether extract was according to AOAC (2006; method 920.39), using an ANKOM XT15 extraction system (ANKOM Technology, Macedon, NY, USA). Non-fiber carbohydrates (NFC) were calculated according to equation: NFC (%) = 100 − (%

NDF + % CP + % EE + % ash). Calculation of coastcross haylage and concentrate TDN was performed according to the equations proposed by NASEM (2016).

Rumen fluid was collected on d 0 (immediately prior to the beginning of the experimental period and 1st treatment offer), 28, 56, 84, 112, and 140 of the experimental period at 0, 6 and 12 hours after feeding the concentrate and, combined as a pool for SCFA analysis. The SCFA concentration was determined according to Ferreira et al. (2016) and ammonia nitrogen according to Chaney and Marbach (1962) with a colorimetric method, adapted for a microplate reader (EON, BioTech Instruments, Winooski, VT, USA) with a 550 nm absorbance filter.

Concurrently with the rumen fluid sampling the blood samples were collected 6 hours after feeding for urea and glucose determination. Blood samples were collected from each steer via coccygeal venipuncture into 4-mL vacutainer tubes with glycolytic inhibitor and anticoagulant K3EDTA (Vacuette, Greiner Bio-One, Americana, SP, Brazil). Blood samples were centrifuged (2000×g at 4°C for 15 min), and serum was harvested into 1.5-mL tubes (Eppendorf AG, Sao Paulo, SP, Brazil) and refrigerated at -20°C until analysis. The blood parameters were determined in the Automatic System for Biochemistry - Model SBA-200 (CELM, Barueri, SP, Brazil). Commercial kits from Labtest Diagnostica SA (Lagoa Santa, MG, Brazil) were used for determination of serum glucose (Ref. 133-1 / 500), and urea (PUN) (Ref. 104).

EXPERIMENT 2

Animal, housing, and Experiment design

One hundred and sixty Nellore (*Bos indicus*) yearling bulls were assigned to a randomized complete block design (n = 10), according to their initial shrunk BW (after 14 h of feed and water restriction; 212 ± 3.1 kg and age = 16 ± 3.0 mo). The experimental period lasted 140 d, divided into 5 periods of 28 d each. Bulls were kept in a covered feedlot (10 pens per treatment; 4 bulls per pen of 18m²) with a concrete floor, feed bunk, and waterer.

Animals Management and Treatments

Bulls were fed daily and diets (Table 1) were composed of 96% of chopped coastcross haylage [Cynodon dactylon (L.) Pers] and 4% of concentrate (50% ground corn and 50% ground citrus pulp dry), used as a delivery vehicle for the additives. The coastcross haylage was chopped using a vertical mixer (Mixer VM8B, DeLaval International AB, Tumba, Sweden) for about 15 min (Final particle length of: 60.1 ± 1.8% > 19 mm; 23.0 ± 1.0% > 8 mm; 8.9 ± 1.7% > 4 mm; and 8.0 ± 1.3% on bottom sieve). The feed additives were mixed into the concentrate and offered to each pen individually. Bulls promptly consumed additives and concentrate within 20 min after feeding and then the haylage was offered. Calculation of coastcross haylage and concentrate TDN was performed according to the equations proposed by NASEM (2016).

Sample collection, laboratory Analyses, and Measurements

To calculate average daily gain (**ADG**) and gain:feed (**G:F**), bulls were individually weighed on days 0, 28, 56, 84, 112 e 140 (final days of each period) after 14 h of feed and water restriction. The feed was recorded daily and orts monthly to determine the dry matter intake (**DMI**).

STATISTICAL ANALYSIS

Experiment 1

Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC, USA), with the period (days 28, 56, 84, 112 and 140) as repeated measures. Treatment and periods were included in the model as fixed effects and block as a random effect. Steer was the experimental unit. The repeated term was the animal with the treatment as the subject. Treatment means were calculated using the LSMEANS option. The effect of diet × period interaction was defined by the *F*-test. Tukey test 5% was used for comparisons. When the data were analyzed, $P \leq 0.05$ was considered significant.

Experiment 2

Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC, USA), with the period (days 28, 56, 84, 112 and 140) as repeated measures. The pen was the experimental unit, dietary treatment and period were a fixed effect, and the block was considered a random effect. Treatment means were calculated using the LSMEANS option. The effect of diet × period interaction was defined by the *F*-test. Tukey test 5% was used for comparisons. When the data were analyzed, $P \leq 0.05$ was considered significant.

RESULTS

Experiment 1

The average daily intake of feed additive was 13.26 ± 0.08, 20.42 ± 0.14, and 20.61 ± 0.15 mg/kg DM to treatments NAR, LAS, and VM, respectively (Fig. 1). The inclusion of feed additives did not affect the DMI ($P = 0.46$), and consequently, did not affect the nutrients intake ($P > 0.05$; Table 2). In addition, there was no difference in nutrient digestibility among diets ($P > 0.05$). However, a day effect was observed to nutrient composition ($P < 0.01$) and nutrient digestibility ($P < 0.01$).

There was an interaction between treatment x day for total SCFA ($P < 0.01$; Fig. 2), acetate ($P < 0.01$; Fig. 3), propionate ($P < 0.01$; Fig. 4), A:P ratio ($P < 0.01$; Fig. 5) and butyrate ($P = 0.03$). The treatment NAR had the greatest total SCFA values on days 84 and 112. The acetate concentration was lowest to NAR on days 28, 56, 112, and lower on day 140 to NAR in relation to CON and LAS. The treatment NAR had the greatest propionate concentration and lowest A:P ratio values on days 28, 56, 112 and 140 when compared to other treatments. A cross-over interaction was found on butyrate, what means there was no treatment effect but, an interaction treatment x day ($P = 0.03$). On 140d, the treatment NAR had the lower value compared to VM ($P < 0.01$). Besides, the inclusion of VM had the greatest ruminal pH ($P = 0.03$) when compared to other treatments. On the other hand, there was no treatment effect to valerate ($P = 0.95$), isobutyrate ($P = 0.84$), isovalerate ($P = 0.27$), and nitrogen ammonia ($P = 0.15$). No differences were found among treatments on serum glucose ($P = 0.76$) and urea concentration ($P = 0.53$; Table 4).

Experiment 2

There was no treatment x day interaction on final BW, DMI, ADG, and G:F ($P > 0.05$; Table 5). There was a treatment effect on final BW ($P = 0.03$), DMI ($P = 0.03$),

ADG (P = 0.04) and G:F (P = 0.05). Narasin supplementation increased DMI compared with other treatments (P = 0.03). The treatment NAR had a greater ADG (P = 0.04) than CON and VM and was similar to the LAS. In turn, the animals that received LAS presented a similar ADG to those of the other treatments. Consequently, the treatment NAR had the greatest final BW than others. The G:F was greater for NAR than CON and VM. Besides, LAS supplementation increased the G:F compared with CON, with no significant difference compared to VM.

DISCUSSION

There was no treatment effect on nutrient digestibility in Exp. 1. In vivo effects on digestibility with ionophores have been minimal (Ricke et al., 1984) or greater (Wedegaertner and Johnson, 1983). The literature data show no effects on nutrient digestibility with monensin (Bell et al., 2017), and virginiamycin (Goulart, 2010) on high-forage diets. However, trials with narasin inclusion to wethers showed a linear increase on fiber digestibility (Polizel et al., 2016b) using 0, 8, 16, 24 and 32 mg/kg DM of narasin. Comparison of the findings with those of other studies confirms the majority ionophore effect is on rumen environment, altering the stoichiometry, increasing energetic efficiency on rumen fermentation (Tedeschi et al., 2003) and increasing propionate production (Beauchemin et al., 2008).

The ruminal fiber digestion is depressed when ruminal pH declines below 6.2 (Grant and Mertens, 1992). In accordance with the present results, previous studies have demonstrated that an increase in ruminal digestibility of the fiber may be associated mainly with high-concentrate diets, where the inclusion of the ionophore improves the ruminal environment, with the increase of the pH, consequently not harming bacteria that digest the fiber, contrary to what occurs with high-forage diets when there is no pH depression. For these reasons, any potential benefits of ionophore and no-ionophore antibiotics supplementation on ruminal fiber digestion might be expected to be small. The literature indicates that the influence on ruminal or post-ruminal nutrient digestion has not been consistent (Nagaraja et al., 1997).

The modulating effect of narasin on ruminal fermentation shows that its mechanisms of action are similar to those of other ionophores. Several reports have shown ionophore modulating rumen environment through action against Gram-positive bacteria (Berg, Hamill, 1978), altering microorganism population, fermentation products and

decreasing methane (Russell and Strobel, 1989; McGuffey et al., 2001; Guan et al., 2006), consequently greater production of total SCFA and propionate (Ellis et al., 2008; Ellis et al., 2012). These changes normally result in increased energy retention and animal performance (Duffield et al., 2012).

The narasin is a feed additive that has recently been used for beef cattle, but it has long been pointed out as an effective antimicrobial in the rumen environment (Nagaraja et al., 1987). Data from metabolism experiments have demonstrated that this molecule is capable of improving ruminal fermentation, increasing the concentrations of propionate and total SCFA and reducing A:P ratio (Polizel et al., 2016a; Polizel et al., 2018). These results are according to the findings of Nagaraja et al. (1987), where lower doses of narasin, about threefold less, resulted in greater ruminal propionate concentration when compared to monensin and lasalocid.

Regarding lasalocid, in a meta-analysis, Golder and Lean (2016) showed an increase in total SCFA concentration, propionate and ammonia compared to the control. However, sorting studies only with high-forage diets (5 studies), in four there was an increase in total SCFA and only one study increased propionate. The doses used were 100 mg/d (2 trials) and 200 mg/d (3 trials). A possible explanation for this might be that ionophore action is dose dependent, where the high or low dose impairs rumen fermentation (Bretschneider et al., 2008; Ellis et al., 2012). In the present study, the dose was 20 mg/kg DM (About 136 mg/d, close to the doses shown in the meta-analysis) as recommended to grazing cattle (Lasalocid 20 mg/kg DM; Zoetis, Sao Paulo, SP, Brazil). Based on results, we assume that this could not be the better dose to cattle fed with high-forage diets.

Virginiamycin is an antibiotic non-ionophore that is active against Gram-positive bacteria through impairing its protein synthesis, resulting in the inhibition of

multiplication an eventually cellular death (Cocito, 1979). The primary effect of virginiamycin is on the concentration of lactic acid in the rumen when ruminants are fed with high-energy diets, increasing ADG, improving ruminal health and reducing the risk of rumen acidosis and hepatic abscesses (Rogers et al., 1995; Tedeschi and Gorocica-Buenfil, 2018). The experimental data are rather controversial, and there is no general agreement about the efficacy of this molecule in high-forage diets at the recommended dose. On grazing trials, there was no effect on rumen parameters when compared to control (Goulart, 2010). More recent arguments against virginiamycin have been made because it is an antibiotic of the class streptogramins, a class shared between human and animals. Therewith, its use as a growth promoter in production animals has been dispensed recently by World Health Organization (Brown et al., 2017).

Surprisingly, no significant differences between treatments were found in values of acetate, propionate, and A:P ratio on day 84. The haylage was bought on bale shape from a local seller, consequently, a variation on chemical composition was expected due to physiological changes in plant development. Sorting the diet in periods, the 28d CP was about 8.4% versus 13.4% of 84d. Besides that, the particle length was different between 28d versus 84d, which 84d had the lower particle size. The day 84 coincided with the regrowth of new grass, which explains the greater quality and smaller particle size (chopped easy). With this finding, we hypothesize that narasin has a greater effect on low-quality forage. However, further research should be conducted to investigate responses to narasin for cattle fed high-forage diets of varying quality. In literature, monensin appears to promote a biological advantage for cattle grazing bermudagrass of low quality (Rouquette et al., 1980). Beyond that, Potter et al. (1976) speculated that feeding monensin to cattle on higher quality forage diets may give a slightly difference response.

There was no treatment effect on the ammonia N, despite literature data show ionophore decreasing rumen ammonia concentration because of decreasing rumen proteolyze (Russell and Martin, 1984) and reducing the amino acid deamination (Chen and Russel, 1991). A possible explanation for these results may be the effect of ionophores on ammonia N depends upon the diet. The greatest response would be expected when dietary protein is not excessive and is supplied in a soluble form likely to be fermented rapidly in the rumen (Hanson and Klopfenstein, 1979). Classical studies have shown that ionophore additives are able to reduce protein degradation in the rumen, reducing the rumen concentration of ammonia, known as a "ruminal protein sparing" (Poos et al., 1979; Russell and Martin 1984; Russell and Strobel, 1989; Rogers et al. 1997). However, in the present study no differences were observed between treatments. Maybe the effect of the additives is dependent on the type of diet, since in experiments in which the animals received forage-based diets, there was no effect of the additives on ruminal ammonia concentration (Davenport et al., 1989; Coe et al., 1999; Bell et al., 2017).

The increases in rumen propionate concentration may result in an increase in glucose concentration through the increase in hepatic gluconeogenic flux (Duffield et al., 2008). However, this effect was not observed in the present study, in which the use of narasin increased the proportion of propionate, despite, did not alter the concentration of serum glucose. In agreement with these results, Bohnert et al. (2016) reported no effects of monensin supplementation on plasma concentrations of glucose in beef steers and late-gestating cows consuming low-quality forage.

Serum urea concentration is associated with ruminal ammonia concentration (Broderick and Clayton, 1997). The decrease in the proteolyze and deamination process reduce the nitrogen ammonia concentration in the rumen (Russell and Martin, 1984; Chen

and Russel, 1991), resulting in lower absorption and consequently lower blood urea concentration. In the present study, the feed additives did not alter the nitrogen ammonia concentration, thus explaining the absence of effect on the plasma concentration of urea.

With respect to performance, the effectiveness of ionophores and non-ionophore antibiotics is attributed principally to alterations in ruminal fermentation (Nagaraja et al., 1997). As mentioned in the literature, in high-concentrate diets, ionophores generally depress DMI, but ADG is increased, or unaffected and G:F is improved. In high-forage diets, ionophores do not reduce DMI but ADG is increased, thus resulting in improved G:F.

The performance trials with narasin on high-forage diets have shown an increase in the ADG (Silva et al., 2015; Polizel et al., 2017; Polizel et al., 2018) and did not reduce DMI when fed in mineral mixture compared to control (Silva et al., 2015). On the Exp. 2, NAR increased final BW and DMI when compared to other treatments. Also, the treatment NAR increased ADG and G:F, when compared to control and VM and, LAS increased G:F when compared to control. In summary of 12 trials conducted in Brazil to evaluate the effect of virginiamycin supplementation of grazing cattle on ADG, Araújo et al. (2016) showed that only 6 trials virginiamycin had greater ADG than control, with an average dose of 111.60 ± 12.87 mg/d.

Despite this, Cappellozza et al. (2018) evaluating narasin inclusion on mineral salt or protein-energetic supplement, did not find reduce supplement intake, moreover, the narasin inclusion on mineral salt increased the frequency of visits to the feeder. The authors suggest narasin inclusion like a viable alternative to improve supplement intake and consequently grazing cattle performance. To the best of our knowledge, trials with narasin in high-concentrate diets also demonstrated no negative effect on DMI (Gobato

et al., 2017). Probably this is a characteristic of narasin, due to a no interaction between DMI and diet type in several studies.

In the present study, the ADG increase was 19.03% greater to NAR as compared to control, besides, G:F was 15.65% greater than control. Regarding LAS, G:F was 12.81% greater than control. Other studies with narasin on high-forage diet showed similar results (Silva et al., 2015: 16.49% ADG; Polizel et al., 2017: 14.26% ADG; Polizel et al., 2018: 13.14% ADG), at the same dose of 13 mg/kg DM. These results are close to shown in studies with monensin on high-forage diets (Potter et al., 1976), where the ADG improve was 16% greater than control. In a summary of ionophores effects in high forage diet, ADG increased by 12.1% with monensin and 10.3% with lasalocid compared to control, with no changes on DMI by either monensin or lasalocid (Bretschneider et al., 2008).

According to Delfino et al. (1988), the main way by which lasalocid improved G:F is by increasing metabolizable energy (ME) density of the diet. In diets with ionophore, molar proportions of propionate increase, and acetate and butyrate decrease, improving ruminal fermentation with less methane produced. So, ME and net energy (NE) values of feeds should increase when ionophores are consumed (NASEM, 2016). The Beef Cattle Nutrient Requirements Model committee (NASEM, 2016), recommends that ME be increased by 2.3% for monensin and 1.5% for lasalocid. There are no available dates, but probably narasin do the same way because the treatment NAR increased DMI, ADG, and G:F as well.

This study has shown that narasin increases propionate, total SCFA and decreases the acetate and A:P ratio. Furthermore, narasin improves performance and LAS improves feed efficiency of yearling bulls fed a high-forage diet. No evidence of benefits was found to support the use of VM to cattle. The findings of this research provide insights for

narasin as an important feed additive to be used on high-forage diets to beef cattle. Further research should be undertaken to investigate the rumen microbiome and effects on varying quality forage using narasin.

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TABLES

Table 1. Ingredients and chemical composition of experimental diets (experiment I and II)

Item[1]	Experiment I		Experiment II	
	Ingredients		Ingredients	
	Coastcross haylage	Concentrate[2]	Coastcross haylage	Concentrate[3]
DM	41.5	88.6	59.7	87.4
CP, % DM	11.6	10.6	12	7.8
NDF, % DM	64.6	37.2	62	16.9
ADF, % DM	34.4	3.5	32.4	3.8
Ash, % DM	9.9	3.2	6.8	4.4
EE, %DM	2.05	2.4	2.10	2.6
NFC, %DM	11.85	74	17.1	72
TDN[4], % DM	55.4	75.1	55.4	78.8
NEm[4], Mcal/kg	1.15	1.78	1.15	1.90
NEg[4], Mcal/kg	0.59	1.15	0.59	1.26

[1] DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein; EE: Ether extract; NFC: non-fiber carbohydrate; TDN: total digestible nutrients; NEm: net energy of maintance; NEg: net energy of gain. Nonfiber carbohydrates (NFC) were calculated according to equation: NFC (%) = 100 − (% NDF + % CP + % EE + % Ash).

[2]Concentrate: 50% ground corn and 50% ground soybean hulls.

[3]Concentrate: 50% ground corn and 50% ground citrus pulp dry.

[4]Calculated composition using tabular values (NASEM, 2016).

Table 2. Intake and apparent digestibility of nutrients in steers receiving the experimental diets

	Treatments[2]					*P* Value[4]		
Item[1]	CON	NAR	LAS	VM	SEM[3]	Treatment	Day	T x D
Intake, kg/d								
DM	6.50	6.99	6.61	6.62	1.28	0.46	<0.01	0.65
OM	5.77	6.37	5.95	5.97	1.27	0.33	<0.01	0.32
NDF	4.17	4.49	4.24	4.27	0.82	0.43	<0.01	0.57
ADF	2.20	2.42	2.24	2.25	0.48	0.31	<0.01	0.71
CP	0.75	0.82	0.77	0.77	0.14	0.33	<0.01	0.49
Digestibility, %								
DM	54.94	57.29	55.76	55.35	1.12	0.48	<0.01	0.36
OM	56.99	59.21	57.84	57.48	1.17	0.36	<0.01	0.88
NDF	61.49	64.18	62.67	61.79	1.15	0.34	<0.01	0.73
ADF	60.84	62.41	60.93	60.48	1.29	0.72	<0.01	0.51
CP	55.79	57.94	57.71	57.87	1.16	0.48	<0.01	0.82

[1] DM: dry matter; OM: organic matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein.
[2]CON: no feed additives; NAR: inclusion of 13 mg/kg DM of narasin; LAS: inclusion of 20 mg/kg DM of lasalocid; VM: inclusion of 20 mg/kg DM of virginiamycin.
[3]SEM: standard error of the mean.
[4]*P* Value for Treatment, Day and Treatment x Day.

Table 3. Ruminal short chain fatty acids (SCFA), ammonia and ruminal pH in steers receiving the experimental diets

	Treatments[1]					*P* Value[3]		
Item	CON	NAR	LAS	VM	SEM[2]	Treatment	Day	T x D
Rumen pH	6.85[b]	6.79[b]	6.84[b]	6.98[a]	0.04	0.03	<0.01	0.19
N ammonia, mg/dl	4.18	4.28	4.25	4.87	0.77	0.11	<0.01	0.17
Total SCFA, mM	87.15[b]	102.51[a]	90.94[b]	90.85[b]	3.6	0.03	<0.01	0.04
SCFA, mM/100mM								
Acetate	74.91[a]	73.14[b]	74.61[a]	74.91[a]	0.38	<0.01	<0.01	<0.01
Propionate	14.53[b]	16.28[a]	14.84[b]	14.49[b]	0.22	<0.01	<0.01	<0.01
Butyrate	7.8	7.75	7.62	7.71	0.14	0.78	<0.01	0.03
Isobutyrate	0.87	0.87	0.88	0.89	0.02	0.84	<0.01	0.47
Valerate	0.87	0.85	0.85	0.88	0.08	0.95	<0.01	0.87
Isovalerate	1.05	1.1	1.11	1.09	0.02	0.27	<0.01	0.06
Acetate:propionate	5.18[b]	4.52[a]	5.04[b]	5.20[b]	0.06	<0.01	<0.01	<0.01

[1]CON: no feed additives; NAR: inclusion of 13 mg/kg DM of narasin; LAS: inclusion of 20 mg/kg DM of lasalocid; VM: inclusion of 20 mg/kg DM of virginiamycin. Means in the same row followed by different letters differ by Tukey's test ($P \leq 0.05$).
[2]SEM: standard error of the mean.
[3]*P* Value for Treatment, Day and Treatment x Day.

Table 4. Serum glucose and urea in steers receiving the experimental diets

	Treatments[1]					*P* Value[3]		
Item	CON	NAR	LAS	VM	SEM[2]	Treatment	Day	T x D
Glucose mg/dL	69.37	69.30	68.63	70.53	1.52	0.76	<0.01	0.51
Ureia mg/dL	32.46	33.74	32.11	34.56	1.36	0.53	<0.01	0.60

[1]CON: no feed additives; NAR: inclusion of 13 mg/kg DM of narasin; LAS: inclusion of 20 mg/kg DM of lasalocid; VM: inclusion of 20 mg/kg DM of virginiamycin.
[2]SEM: standard error of the mean.
[3]*P* Value for Treatment, Day and Treatment x Day.

Table 5. Performance in yearling bulls receiving the experimental diets

	Treatments[2]					*P* Value[4]		
Item[1]	CON	NAR	LAS	VM	SEM[3]	Treatment	Day	T x D
BW, kg								
Initial	214	211	210	215	3.1	0.61		
Final	273.9^{b}	287.8^{a}	277.1^{b}	275.7^{b}	3.4	0.03	<0.01	0.20
DMI, kg	5.26^{b}	5.69^{a}	5.16^{b}	5.11^{b}	0.14	0.03	<0.01	0.99
ADG, kg	0.451^{b}	0.557^{a}	0.498^{ab}	0.459^{b}	0.03	0.04	<0.01	0.16
G:F	0.0803^{c}	0.0952^{a}	0.0921^{ab}	0.0846^{bc}	0.0044	0.05	<0.01	0.40

[1]Body weight (BW), average daily gain (ADG), dry matter intake (DMI) and gaind:feed (G:F) of bulls fed the experimental diets.
[2]CON: no feed additives; NAR: inclusion of 13 mg/kg DM of narasin; LAS: inclusion of 20 mg/kg DM of lasalocid; VM: inclusion of 20 mg/kg DM of virginiamycin. Means in the same row followed by different letters differ by Tukey's test ($P \leq 0.05$).
[3]SEM: standard error of the mean.
[4]*P* Value for Treatment, Day and Treatment x Day.

FIGURES

Figure 1. Average daily intake of feed additives on metabolism. 1A: average daily intake of NAR (13.26 ± 0.08 mg/kg DM). 1B: Average daily intake of LAS (20.42 ± 0.14 mg/kg DM). 1C: Average daily intake of VM (20.61 ± 0.15 mg/kg DM).

Figure 2. Effect of treatments on the total SCFA in the ruminal fluid of steers consuming high-forage diet. CON: no feed additives; NAR: inclusion of 13 mg/kg DM of narasin; LAS: inclusion of 20 mg/kg DM of lasalocid; VM: inclusion of 20 mg/kg DM of virginiamycin. Treatment x day interaction (P <0.01). *Differences between treatments at the $P \leq 0.05$, where NAR was greater than other treatments.

Figure 3. Effect of treatments on the acetate in the ruminal fluid of steers consuming high-forage diet. CON: no feed additives; NAR: inclusion of 13 mg/kg DM of narasin; LAS: inclusion of 20 mg/kg DM of lasalocid; VM: inclusion of 20 mg/kg DM of virginiamycin. Treatment x day interaction (P <0.01). The comparison between the treatments and the letters should be done within each day ($P \leq 0.05$).

Figure 4. Effect of treatments on the propionate in the ruminal fluid of steers consuming high-forage diet. CON: no feed additives; NAR: inclusion of 13 mg/kg DM of narasin; LAS: inclusion of 20 mg/kg DM of lasalocid; VM: inclusion of 20 mg/kg DM of virginiamycin. Treatment x day interaction (P <0.01). The comparison between the treatments and the letters should be done within each day ($P \leq 0.05$).

Figure 5. Effect of treatments on the acetate:propionate ratio in the ruminal fluid of steers consuming high-forage diet. CON: no feed additives; NAR: inclusion of 13 mg/kg DM of narasin; LAS: inclusion of 20 mg/kg DM of lasalocid; VM: inclusion of 20 mg/kg DM

of virginiamycin. Treatment x day interaction (P <0.01). The comparison between the treatments and the letters should be done within each day ($P \leq 0.05$).

Figure 1.

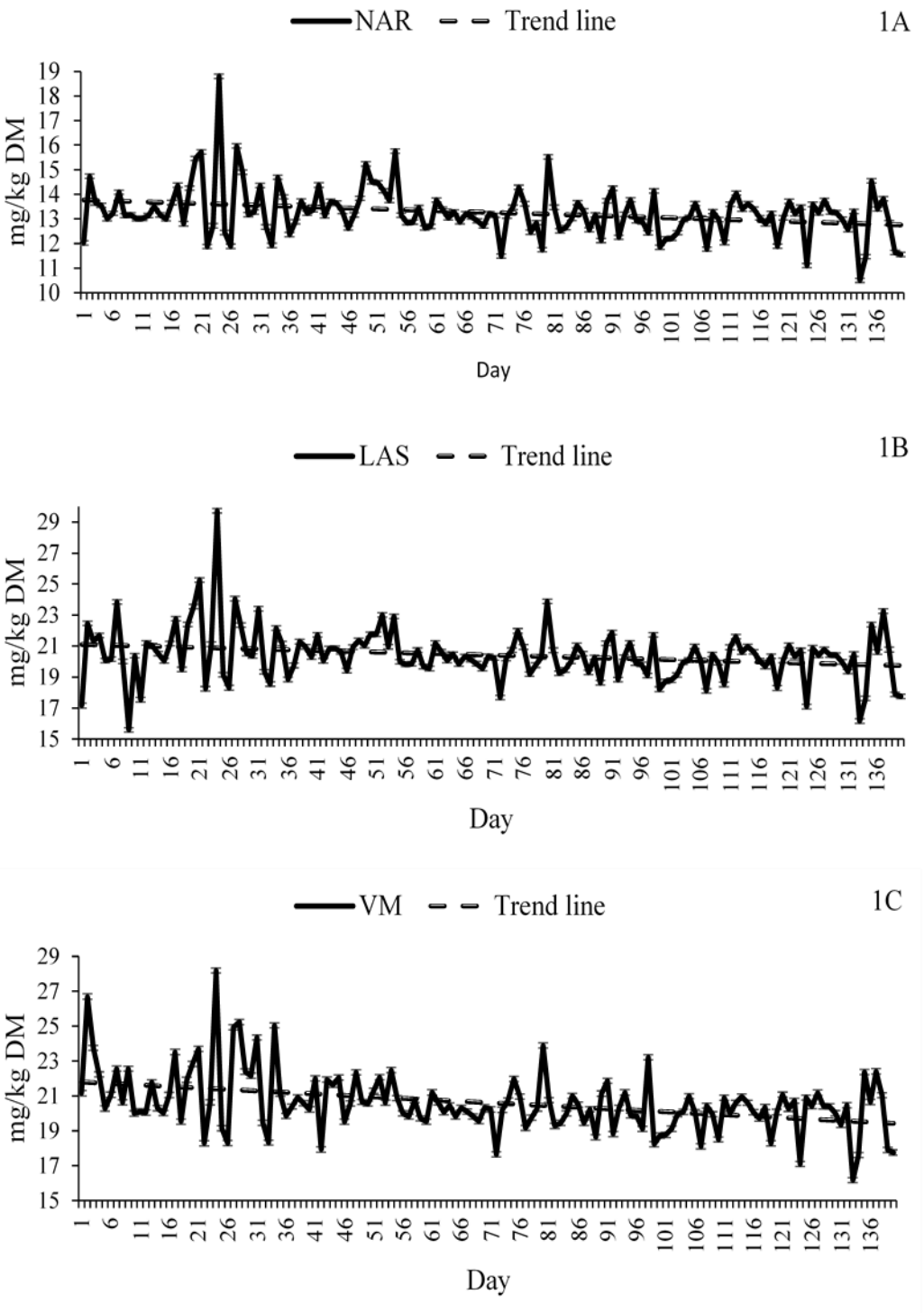

NAR
Trend line
1A
mg/kg DM
Day
LAS
Trend line
1B
mg/kg DM
Day
VM
Trend line
1C
mg/kg DM
Day

Figure 2.

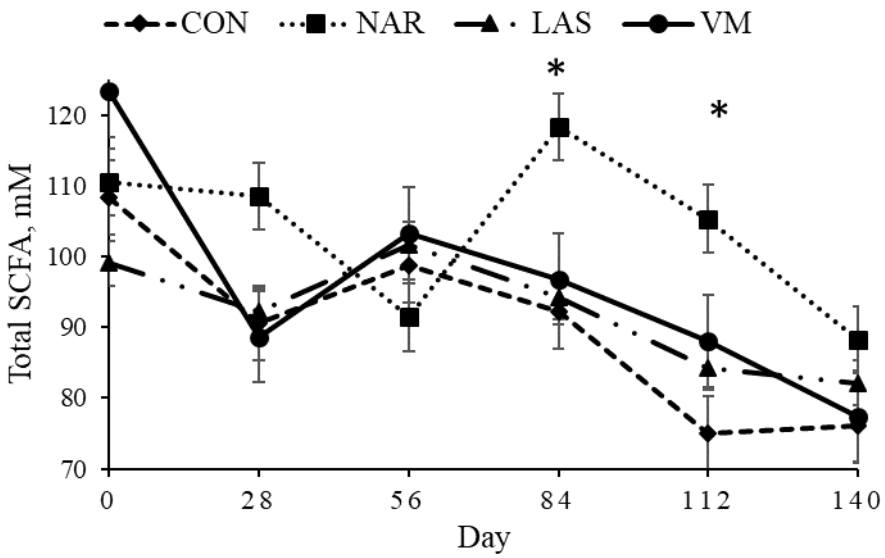

CON
NAR
LAS
VM
*
*
Total SCFA, mM
120
110
100
90
80
70
0
28
56
84
112
140
Day

Figure 3.

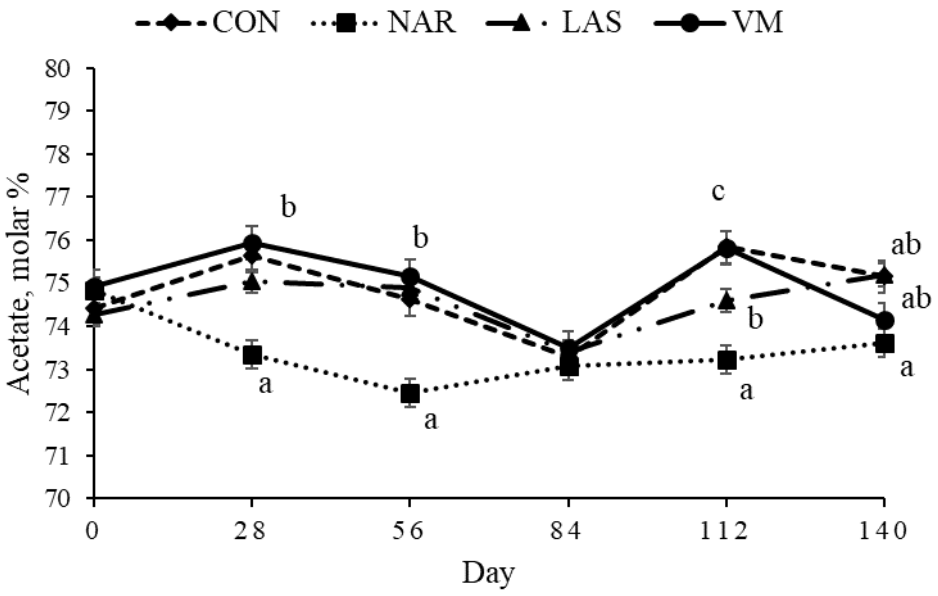

CON
NAR
LAS
VM
Acetate, molar %
Day
80
79
78
77
76
75
74
73
72
71
70
0
28
56
84
112
140
b
b
c
ab
ab
b
a
a
a

Figure 4.

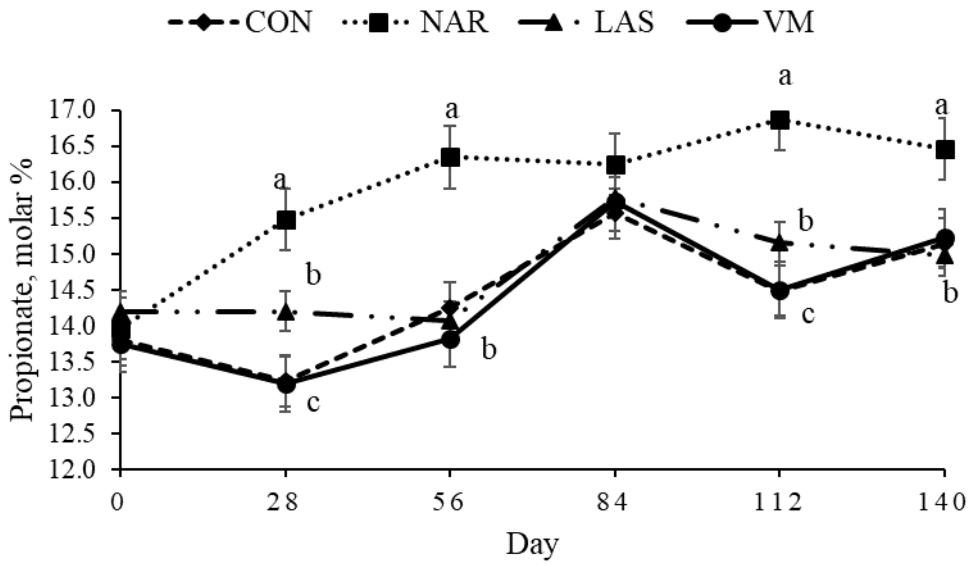
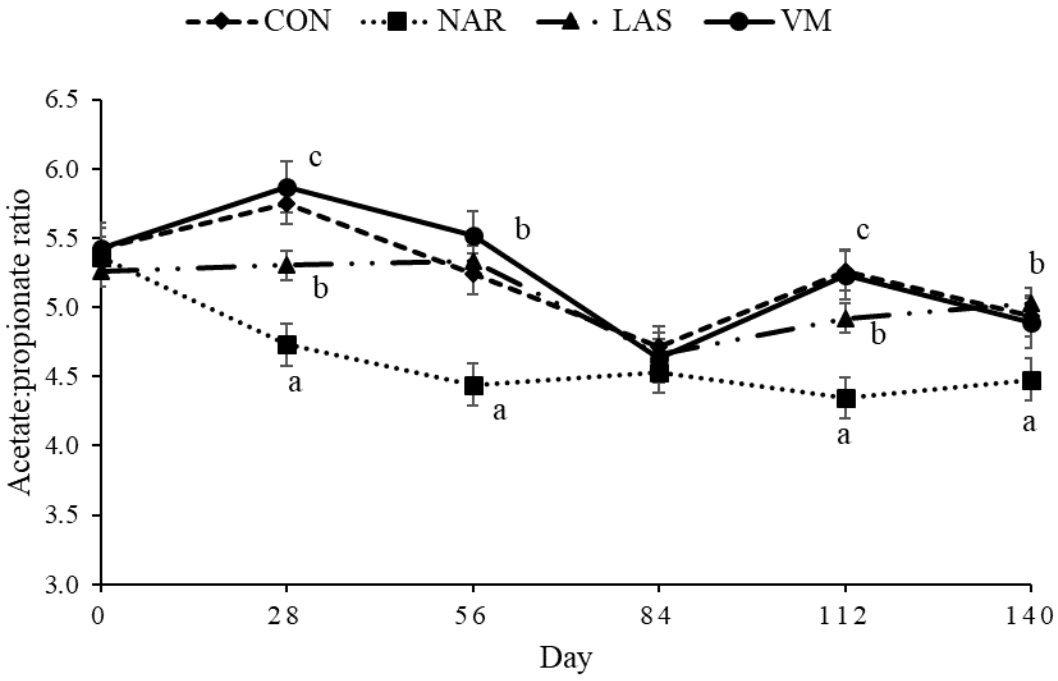

CON
NAR
LAS
VM
Propionate, molar %
17.0
16.5
16.0
15.5
15.0
14.5
14.0
13.5
13.0
12.5
12.0
0
28
56
84
112
140
Day
a
a
a
a
b
b
b
b
c
c

Figure 5.


CON
NAR
LAS
VM
Acetate:propionate ratio
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0
28
56
84
112
140
Day
c
b
b
a
a
c
b
a
b