ARNALDO CINTRA LIMEDE

Long-term use of narasin, salinomycin and flavomycin for Nellore steers fed with high-forage diets: ruminal parameters, apparent nutrient digestibility, and performance

Pirassununga

2020

ARNALDO CINTRA LIMEDE

Long-term use of narasin, salinomycin and flavomycin for Nellore steers fed with high-forage diets: ruminal parameters, apparent nutrient digestibility, and performance

Dissertation submitted to the Post-graduate Program in Nutrição e Produção Animal of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the master's degree in Sciences.

Department:

Nutrição e Produção Animal

Area:

Nutrição e Produção Animal

Advisor:

Prof. Alexandre Vaz Pires, Ph.D.

In accord:_____

Advisor

Pirassununga
2020

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO

(Biblioteca Virgínia Buff D'Ápice da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo)

T. 3962
FMVZ

Limeda, Arnaldo Cintra
Long-term use of narasin, salinomycin and flavomycin for Nellore steers fed with high-forage diets: ruminal parameters, apparent nutrient digestibility and performance / Arnaldo Cintra Limeda. – 2020.
71 f. : il.

Título traduzido: Uso prolongado de narasina, salinomicina e flavomicina para novilhos Nelores alimentados com dieta de elevado teor de volumoso: parâmetros ruminais, digestibilidade aparente de nutrientes e desempenho.

Dissertação (Mestrado) – Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Nutrição e Produção Animal, Pirassununga, 2020.

Programa de Pós-Graduação: Nutrição e Produção Animal.
Área de concentração: Nutrição e Produção Animal.
Orientador: Prof. Dr. Alexandre Vaz Pires.

1. Aditivos alimentares. 2. Desempenho. 3. Parâmetros ruminais. 4. Digestibilidade. I. Título.

Ficha catalográfica elaborada pela bibliotecária Maria Aparecida Laet, CRB 5673-8, da FMVZ/USP.

CERTIFICADO

Certificamos que a proposta intitulada "Efeito de aditivos sobre parâmetros de fermentação ruminal e sanguíneo, digestibilidade de nutrientes e microbiologia ruminal de novilhos Nelores em dietas de alto volumoso", protocolada sob o CEUA nº 8582080119 (ID 006252), sob a responsabilidade de **Alexandre Vaz Pires** *e equipe; Arnaldo Cintra Limede* - 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 07/03/2019.

We certify that the proposal "Effect of additives about ruminal fermentation and blood parameters, digestibility of nutrients and ruminal microbiology in Nellore young bulls fed high forage diets ", utilizing 192 Bovines (192 males), protocol number CEUA 8582080119 (ID 006252), under the responsibility of **Alexandre Vaz Pires** *and team; Arnaldo Cintra Limede* - 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 03/07/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 02/2019 a 02/2022 Área: Nutrição E Produção Animal

Origem:	Animais provenientes de outros projetos						
Espécie:	Bovinos	sexo:	Machos	idade:	16 a 17 meses	N:	32
Linhagem:	Nelore			Peso:	250 a 280 kg		
Origem:	Animais provenientes de outros projetos						
Espécie:	Bovinos	sexo:	Machos	idade:	24 a 24 meses	N:	160
Linhagem:	Nelore			Peso:	300 a 350 kg		

Local do experimento: O experimento será realizado nas instalações do Laboratório de Nutrição e Reprodução Animal pertencente ao Departamento de Zootecnia da Escola Superior de Agricultura []Luiz de Queiroz[] (ESALQ/USP).

São Paulo, 25 de maio de 2020

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
Vice-Coordenador
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

EVALUATION FORM

Author: Limede, Arnaldo Cintra

Title: **Long-term use of narasin, salinomycin and flavomycin for Nellore steers fed with high-forage diets**: ruminal parameters, apparent nutrient digestibility and performance

Dissertation submitted to the Post-graduate Program in Nutrição e Produção Animal of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Master's degree in Sciences.

Date: _____/_____/_____

Committee Members

Prof. _____

Institution:_____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

Prof. _____

Institution: _____ Decision: _____

DEDICATION

To my family for unconditional support and love. You are my strength.

ACKNOWLEDMENTS

To God and my spiritual guides for protection and for allowing me to get here.

To my grandfather, Arnaldo, for always way with me and be my inspiration and example of light. One day we'll meet.

To my parents, Arnaldo and Simone, for all support and for being my example of love, respect, and faith, and for always trust me.

To my sisters, Mariana and Angela, for friendship, love, and support.

To my nephew, Antonio, for bringing more joy to my days since your arrival.

To my uncles, grandparents and cousins for love, friendship and support.

To my advisor, Alexandre Vaz Pires, for opening the doors and teaching me valuable lessons that I will carry to life.

To my friend and co-advisor, Daniel Polizel, for the lessons, patience and friendship since my first day in master. For the trips, mainly to Londrina, and obvious, for not letting me get lost in the USA.

To my friend Rodrigo Marques for his help, encouragement and friendship

To all my friends of Animal Nutrition and Reproduction Laboratory, José Paulo, Alexandre Miszura, André Xena, Gabriela Bagio, Lairana Sardinha, Matheus Felipe, my old friend Francisco Montanaro, Marcelo Baggio, Kauê Maia, Matheus Carlis, Thamires, and Nathalia, thank you guys, you are phenomenous!

To all my friends of Piracicaba, especially Rodrigo Lemos, Pedro Guerreiro, and Lucas Oliveira for the partnership, singing, Nildo's nights, and many beers.

To employees of Department of Animal Science, especially to Danilo Grajal, for the good laughs.

To the College of Agriculture "Luiz de Queiroz" (ESALQ/USP), for the facilities provided, making it possible to carry out research.

To School of Veterinary Medicine and Animal Science (FMVZ/USP) for the opportunity to be a master's student.

To CAPES for financial support.

Thank you!

“The mind that opens to a new idea never returns to its original size”

- Albert Einstein

RESUMO

LIMEDE, A. C. **Uso prolongado de narasina, salinomicina e flavomicina para novilhos Nelores alimentados com dieta de elevado teor de volumoso:** parâmetros ruminais, digestibilidade aparente de nutrientes e desempenho. 2020. 71 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2020.

Experimento 1: Trinta e dois novilhos Nelore (*Bos indicus*) providos de cânula ruminal foram distribuídos em delineamento de blocos completos casualizados de acordo com o peso corporal em jejum (220 ± 12,6 kg). Os animais foram alimentados diariamente com dietas compostas por 99% de feno pré-secado de coastcross e 1% de concentrado, utilizado como veículo para fornecimento dos aditivos. As dietas experimentais foram: 1) CON – dieta a base de forragem sem inclusão de aditivos, 2) NAR – inclusão de 13 ppm de narasina, 3) SAL – inclusão de 20 ppm de salinomicina e 4) FLA – inclusão de 3 ppm de flavomicina. O período experimental foi de 140 dias, subdivididos em 5 períodos de 28 dias cada. Não houve interação de tratamento × dia para os parâmetros de consumo e digestibilidade. A inclusão de aditivos não afetou o CMS (P = 0,20) e, consequentemente não afetou a ingestão de nutrientes (P > 0,17). De mesma forma não houve diferença na digestibilidade aparente dos nutrientes entre as dietas (P > 0,40). Houve interação entre tratamento × dia (P < 0,01) para relação AcBut:Prop em que o tratamento NAR apresentou valores menores que os demais tratamentos a partir do segundo período experimental (P < 0,01). Ainda, os animais do tratamento narasina apresentaram menores valores de concentração molar de acetato (P < 0,01), e maiores concentrações molares de propionato (P < 0,01), butirato (P < 0,01) e relação Ac:Prop (P < 0,01). O total de ácidos graxos foi menor para os tratamentos SAL e FLA em relação a CON e NAR (P = 0,02). **Experimento 2:** cento e sessenta e quatro novilhos Nelore (*Bos indicus*) foram blocados a partir do peso corporal em jejum (299 ± 2,5) em confinamento experimental com duração de 140 dias, subdividido em 5 períodos de 28 dias cada. Os animais foram alimentados diariamente com dietas contendo 96% de feno pré-secado de coastcross e 4% de concentrado, utilizados como veículo de fornecimento dos aditivos. Os tratamentos utilizados foram os mesmos do experimento anterior. Houve interação tratamento × dia para PC (P < 0,01) e CMS (P = 0,03). Foi observado maior PC para os animais do tratamento NAR a partir do terceiro período experimental (P = 0,03) e apresentaram maior CMS no segundo, quarto e quinto período experimental (P < 0,05).

Em conclusão, a inclusão de 13 ppm de narasina melhora os parâmetros de fermentação ruminal e o desempenho de novilhos em dietas à base de forragem. Não houveram evidências de que o uso de salinomicina e flavomicina sejam eficazes para dietas à base de forragem.

Palavras-chave: Aditivos alimentares, desempenho, digestibilidade, parâmetros ruminais

ABSTRACT

LIMEDE, A. C. **Long-term use of narasin, salinomycin and flavomycin for Nellore steers fed with high-forage diets**: ruminal parameters, apparent nutrient digestibility and performance. 2020. 71 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia. Universidade de São Paulo, Pirassununga, 2020.

Experiement 1: Thirty-two rumen-fistulated steers Nellore steers (*Bos indicus*) were assigned to a randomized complete block design, according to their initial shrunk BW (220 ± 12.6 kg). 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) CON - a forage-based diet without additives, 2) NAR - CON diet plus 13 ppm of narasin, 3) SAL - CON diet plus 20 ppm of salinomycin, or 4) FLA - CON diet plus 3 ppm of flavomycin. The experimental period lasted 140 d, divided into 5 periods of 28 d each. There was no interaction treatment × day for parameters of intake and digestibility. The inclusion of feed additives did not affect the DMI (P = 0.20), and consequently, did not affect the nutrients intake (P > 0.17). In addition, there was no difference in nutrient digestibility among diets (P > 0.40). There is an interaction between treatment x day (P < 0.01) for AcBut:Prop ratio where the NAR treatment had the lowest values from the 56 day (P < 0.01). In addition, animals receiving narasin showed greatest values to acetate (P < 0.01) and higher values to propionate (P < 0.01), butyrate (P = 0.01) and Ac:Prop (P < 0.01). The Volatile Fatty Acids Total was lowest to SAL and FLA treatments (P = 0.02). **Experiment 2:** one hundred and sixty-four Nellore bulls (*Bos indicus*) were blocked by initial shrunk BW (298.95 ± 2.5) in a 140-d feedlot trial, divided into 5 periods of 28 d each. 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 treatments used were the same as in the previous experiment. There was an interaction between treatment x day for BW (P < 0.01) and Dry Matter Intake (DMI; P = 0.03). Animals of treatment NAR showed greatest BW from 84th day of the experiment (P = 0.03) and had higher DMI in the second, fourth, and fifth period of study (P < 0.05). In conclusion, the inclusion of 13 ppm of NAR improves the ruminal fermentation parameters and the performance of bulls in forage-based diets. There were no evidence that SAL and FLA are effective to forage-based diets.

Keywords: feed additives, digestibility, performance, ruminal parameters

LIST OF FIGURES

LIST OF TABLES

SUMARY

1. INTRODUCTION

Influenced by the favorable climate, the beef cattle industry in Brazil is predominant of animals on pasture. In the last year, 85,94% of slaughtered animals were on the no-feedlot system (ABIEC, 2020). In addition to climate, the area of pastures is relevant: 45% of rural areas are pasture in Brazil, totalizing approximately 160 million hectares (IBGE, 2017). According to Silva et al. (2016), when well used, these systems can produce a high-quality protein with sustainable, what shows the high potential of improving one of the most relevant beef cattle industry of the world.

However, diets containing high levels of forage have nutritional particularities. The nutrient intake of these diets is regulated mainly by the physical limitation (Mertens, 1994), once that the content of neutral detergent fiber (NDF) on diets is negatively related to digestibility (Buxton et al., 1995), causing an accumulation of food on rumen and generating signals to stop the meal (Allen, 1996). In addition, the energy efficiency of this diets is lower than high-concentrate diets due to higher acetate production (Owens and Balsan, 2016) and consequently increasing the production of hydrogen (H_2) and carbon dioxide (CO_2), substrates to methane production (Kozloski, 2016; Owens and Balsan, 2016). The methane losses can be representing 2 to 12% of dietary energy (Russell and Strobel, 1989; McGuffey et al., 2001).

Antibiotics growth-promoters as ionophores and non-ionophores are tools able to improve the energy use of the diet and performance of beef cattle in forage-based diets (Bretschneider et al., 2008) through of changes on rumen microbiota (Bergen and Bates, 1984; Russell and Strobel, 1989, Russell et al., 1991). However, few studies of these molecules are directed to these diets (Tedeschi et al., 2003) as well as their effects on long-term administration.

The aim of this study was to evaluate the long-term effect of narasin, salinomycin, and flavomycin on ruminal parameters, apparent nutrient digestibility and performance of steers Nellore fed with high-forage diets.

2. LITERATURE REVIEW

2.1. Forage-based diets

According to the last census, the Brazilian Association of Beef Export Companies (ABIEC, 2019) estimates about 88% of herd (beef or dairy cattle) are raised in162,2 million hectares of pasture in Brazil. In addition, there are production systems where the pasture is exclusive, such as swamp areas (Pantanal) and cow-calf systems. This scenario is only possible due to most of the regions of Brazil are favorable to the high production of tropical forages mainly in the summer period. When well used, this system is an efficient and sustainable way to produce a high-quality protein with a minimum environmental impact (Silva et al., 2016). Although very used, the available energy and metabolizable protein content in forages are often low relative to animal requirements (Buxton et al., 1995).

The nutrient intake of grazing cattle is affected by multiple factors that interact with each other including forage allowance (Drescher et al., 2006), the forage sward structure (Da Silva et al., 2013), grazing management including the ideal time to harvest (light interception concept; Hodgson, 1990; Dorea et al., 2020), and the grazing pressure (Costa et al., 2019). Intrinsic to intake, the quality and the chemical composition of forage are linked to a nutrient intake. In the summer, there is a variation of 14 to 21% of crude protein (CP) and 60 to 63% of NDF in the most used tropical forages in Brazil, including *Brachiaria spp.*, *Pannicum spp.*, *Cynodon spp.* and *Pennisetum spp.* (Lopes, 2011). Soares Filho et al., (2002) evaluated the nutritive value of the mainly tropical forages

cultivate in Brazil for two years, on wet and dry seasons. According to authors, there is influence of season for the crude protein of forages, where in wet season the crude protein content is higher. On the other hand, there is no difference in NDF content.

Although many factors are important, the physical limitation is the most relevant factor to forage-intake (Mertens, 1994) and performance of cattle fed with forage-based diets. The physical limitation occurs when animals eat forage, rich in NDF until the rumen is full. In this compartment, tensions receptor localized primarily on the reticulum and the cranial sac respond to the distention and generates a signal to stop the meal (Allen, 1996). Due to the NDF concentration be negatively related to the digestibility, there are the highest accumulate of forage on the rumen, once the filling effect is determined by the rate of disappearance of forage, digestion, and passage rate (Ellis, 1978; Buxton et al., 1995).

Animals fed with forage-based diets have characteristics on ruminal fermentation. According to Owens and Balasan (2016), the proportion of acetate:propionate:butyrate produced in the ruminal fermentation change from 65:20:15 to 45:40:15 for forage-based diets to concentrate-based diets, respectively. In addition to greater production of acetate, the production of methane increases due to the greater production of hydrogen (H_2), which added to the carbon dioxide there is methane formation by methanogenic bacteria (Kozloski, 2016; Owens and Balsan, 2016). Olijhoek et al. (2018) observed a reduction on the molar concentration of acetate and acetate: propionate ratio, while butyrate was unaffected, resulting on an increase on methane production on average from 25.5 to 32.1 l/kg of dry matter intake (DMI) when the forage:concentrate was altered from 68:32 to 47:53. Up to 12% of dietary energy can be lost by methane eructation (Russell and Strobel, 1989; McGuffey et al., 2001), and added to the lower molar concentration of

propionate, energy efficiency from fermentation is impacted (Russell and Strobel, 1989; McGuffey et al., 2001; Ipharraguerre and Clark, 2003; Weimer et al., 2008).

The addition of concentrate is not the only way to enhance the performance and energy efficiency of ruminants fed with forage-based diets. According to overview of van Gastelen et al. (2019), strategies as a forage type (silage inclusion, tannin-rich forage, or legumes) and feed additives as tannins, 3-nitrooxypropanol, nitrate and garlic oil are efficient to mitigate methane in this diet. Another strategy is the use of antibiotics as growth promoters. Bretschneider et al. (2008) in a review proved that the use of antibiotics ionophores and non-ionophores can improve performance of beef cattle fed with high forage diets. However, in opposition to that, most studies are taken with high-concentrate diets. In compilation made by Tedeschi et al., (2003) with use of monensin to beef cattle, were 263 references to high-concentrate diets and 36 references to pasture.

2.2. Antibiotics Ionophores

Ionophores are carboxylic polyether antibiotics, produced by Streptomyces spp. bacteria that are not used on human or veterinary medicine (McGuffey, 2017). The first ionophore described was the lasalocid in 1951, followed by monensin in 1967 and allowed to beef cattle use in 1975 by FDA. Because the wide acceptance and enhances promoted on animal performance, surge the newest interest on several antimicrobial molecules ionophores and non-ionophores such as avoparcin, laidlomycin, lysocellin, narasin, salinomycin, virginiamycin, and thiopeptin (Nagaraja et al., 1987).

The effects of these molecules on animal performance are similar but may vary depending on dosage, animal, and diet (Tedeschi et al., 2003). With diets containing a high concentration of readily fermentable carbohydrates (concentrate diets), ionophores generally depress the feed intake while the rate of body weight gain is unaffected or improves slightly, improving the feed efficiency (Russell and Strobel, 1989). When

animals are fed with forage-based diets, the feed intake is not decreased and the body weight gain improves, both enhancing the feed efficiency (Bergen and Bates, 1984; Duffield et al., 2012). However, most of the studies with ionophores on ruminant diets are in high-grain feedlot diets (Tedeschi et al., 2003; Ellis et al., 2012; Duffield et al., 2012), needing more studies about pasture or high-forage conditions.

The effects observed on animal performance are reflex of the changes on ruminal microbiota promoted by the presence of these molecules. Initially, it was believed that ionophores act against only Gram-positive bacteria. However, recently researchers showed that Gram-negative bacteria with a cellular wall like Gram-positives are susceptible either (Schären et al., 2017). This selection can inhibit the growth of proteolytic ruminal bacteria, bacteria producer of hydrogen, and formate while succinate and propionate bacteria producers are more tolerant (Russell and Strobel, 1989). In addition, lactate producers and protozoa also inhibited and lactate utilizing bacteria are unaffected (McGuffey et al., 2001). As consequences, the most frequently effects observed on ruminal fermentation are (Bergen and Bates, 1984; Russell and Strobel, 1989; McGuffey et al., 2001; Tedeschi et al., 2003; McGuffey, 2017):

1. Increased efficiency of energy metabolism of rumen bacteria and/or the animal;
2. Up to 30% reduction in rumen methane production;
3. Improve nitrogen metabolism of rumen bacteria and/or the animal;
4. Retardation of digestive disorders resulting from abnormal rumen fermentation;
5. Decrease coccidiosis on feedlot and pasture.

Ionophores act against susceptible bacteria through the change in the concentration of ions intracellular and extracellular (Bergen and Bates, 1984). Each

ionophore has a specific affinity to metal ions (Nagaraja, 1995), and it is directly related with the size of chelation cavity (McGuffey et al., 2001) (Table 1). Although ionophores share a common mode of action, these differences show the variety of the capacity to achieve effective rumen concentration and on the efficiency by which the bacterial changes are induced (Pressman, 1976).

Table 1. Chemical characteristics of mainly Ionophores used on Brazil beef cattle.

Ionophore	Molecular weight	Cation selectivity sequence
Monensin	671	$Na^+ > K^+, Li^+ > Rb^+ > Cs^+$
Lasalocid	591	$Ba^{++}, K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$
Narasin	765	$Na^+ > K^+, Rb^+, Cs^+, Li^+$
Salinomycin	751	$Rb^+, Na^+ > K^+ >> Cs^+, Sr^+, Ca^{++}, Mg^{++}$

Adapted of Nagaraja (1995).
[1] Selectivity sequence of cation flux across bilayer membranes.

The presence of polar and non-polar regions on ionophores facilitate the adherence on bacterial membrane, and once adhered act as antiporters (Pressman, 1976; Russell and Houlihan, 2003). These antiporters bind protons and metal ions (e.g. Na+ and K+) allowing their free transference through the bacterial membrane. The concentration of Na+ outside the cell is 2 to 10-fold greater than K+ concentration inside the cell; the ionophore increases the influx of Na+ and protons and the efflux of K+ causing an osmotic unbalance and the acidification inside of the cell forcing activation of membrane ATPase and transporters to the maintenance of osmotic equilibrium (Russell and Houlihan, 2003; Fig. 1). The junction of these factors de-energy the bacteria cell or disrupts their membrane caused by the greater influx of water (osmotic process), or even

the osmotic pressure incapacity the cell of self-multiplicate (Bergen and Bates, 1984; Russell and Strobel, 1989).

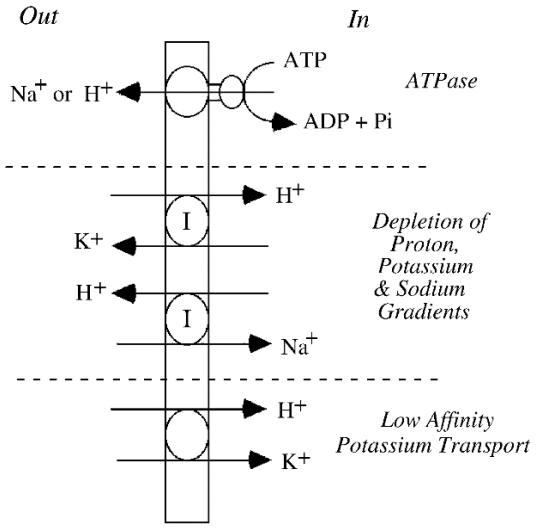


Figure 1. Scheme of the mode of action by ionophores in susceptible bacteria (Adapted of Russell and Houlihan, 2003).

Besides scientific data proving the efficacy of animal performance, the sustainable on the milk and beef chain and the positive impact on the environment, in 1999 the European Union banned the use of ionophores as growth promoters. This decision is sustained on the precaution of the transfer risk of antibiotic resistance through the food animals fed with antibiotics (Klein and Franz, 2005). However, scientific data showed that meat and milk produced by animals fed with ionophores as monensin are safe to human consumption (Donoho, 1984; Wilkinson et al., 1997). More recently, the World Health Organization (2016) did not classify ionophores as important to human health risk, once their use is specific to animal growth promoters. Furthermore, the bacterial resistance to ionophores is related to the cellular wall characteristics (Russell and Houlihan, 2003) and not to biochemist mutations and intrinsic factors of bacteria organism.

2.3. Antibiotics non-ionophores

Antibiotics non-ionophore are used on animal production as therapeutics, metaphylactic, prophylactic, and growth promoters (Aarestrup, 2005). When used as growth promoters, their dose is lower and continuous. According to Nagaraja et al., (1997), antibiotics non-ionophores growth promoters have anti-bacterial activity directed by Gram-positive bacteria and some Gram-negative bacteria improving the energy efficiency of rumen fermentation, decrease methane production, enhance feed efficiency, and the animal performance. Besides, some are used to the prevention of diseases as liver abscess. In addition, there are data on literature that show a reduction in protozoa ruminal population (Edwards et al., 2005).

The non-ionophores antibiotics represent a diverse group differing in chemistry, primary antibacterial spectrum, mode of action inhibition, molecular weight, and ability to be absorbed from de gut (Van Der Merwe et al., 2001). These molecules act against the target inhibit the bacterial growth and replication of targets through peptidoglycan synthesis, ribosome activity inhibits the protein synthesis, DNA replications, mRNA transcription, and membrane stability (Russell and Houlihan, 2003).

In Brazil, virginiamycin and flavomycin are the main non-ionophores used on beef cattle to enhance performance. The flavomycin (synonyms: moenomycin, flaophospholipol, and bambermycin) is a glycolipid antibiotic produced by Streptomyces species including S. bambergiensis, S. ghanaensis, and S. enderensis and actually is only used as a growth-promoting antibacterial in animal feeds (Butaye et al., 2003). Flavomycin inhibits peptidoglycan polymerase through the impairment of the transglycolase activities of penicillin-binding proteins (PBPs). As a result, there is a specific blocking of the formation of peptidoglycan cords and, consequently, failure in the synthesis of the bacterial cell wall (Van Heijenoort, 2001; Butaye et al., 2003).

The growth-inhibitory effect of flavomycin on the pure culture of rumen bacteria were observed in several species of hyper-ammonia producers (HAP) and Gram-positive *Fusobacterium* which has high activity on deamination (Russell et al., 1991; Edwards et al., 2005). However, their effects on ruminal fermentation are unusually observed for beef cattle (Nagaraja et al., 1997), is possible that its effects are mainly at the intestinal level (MacRae et al., 1999; Butaye et al., 2003). In the other hand, Murray et al., (1990) observed in sheep supply with flavomycin an increase on time to animal eat the daily ration, can be a signal to decrease passage rate allowing the enhance on ruminal fermentation, nutrient absorptive capacity, and consequently increase the feed efficiency.

In forage-based diets, data have shown the possible enhance of animals' performance when fed with flavomycin (Bretschneider et al., 2008). However, few trials were designed to study a long-term effect of this molecule, recently data create the hypothesis that the effect of flavomycin is punctual and not persistent caused by bacterial resistance or adaptation (Butaye et al., 2003; Crossland et al., 2017).

Just like ionophores, the flavomycin is not classified within the important group to human health by WHO (2016), due to their use be exclusively as growth promoter since the 90s. Despite the great successful enhance of ionophores on ruminal fermentation and the performance of beef cattle, the potential of antibiotics non-ionophores on these effects cannot be ruled out (Rodrigues, 2016).

3. LITERATURE CITED

ASSOCIAÇÃO BRASILEIRA DAS INDÚSTRIAS EXPORTADORAS DE CARNE (ABIEC). Beef Report – Perfil da Pecuária no Brasil, São Paulo, 2019.

Aarestrup, F. M. 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin. Pharmacol. Toxicol. 96:271–281. doi:10.1111/j.1742-7843.2005.pto960401.x.

Allen, M. S. 1996. Physical Constraints on Voluntary Intake of Forages by Ruminants. J. Anim. Sci. 74:3063–3075. doi:10.2527/1996.74123063x.

Bergen, W. G., and D. B. Bates. 1984. Ionophores: their effect on production efficiency and mode of action. J. Anim. Sci. 58:1465–1483. doi:10.2527/jas1984.5861465x.

Bretschneider, G., J. C. Elizalde, and F. A. Pérez. 2008. The effect of feeding antibiotic growth promoters on the performance of beef cattle consuming forage-based diets: A review. Livest. Sci. 114:135–149. doi:10.1016/j.livsci.2007.12.017.

Butaye, P., L. Devriese, and F. Haesebrouck. 2003. Antimicrobial growth promoters used in animal feed. Clin. Microbiol. Rev. 16:175–188. doi:10.1128/CMR.16.2.175.

Buxton, D. R., D. R. Mertens, and K. J. Moore. 1995. Forage Quality for Ruminants: Plant and Animal Considerations. Prof. Anim. Sci. 11:121–131. doi:10.15232/S1080-7446(15)32575-4.

Costa, D. F. A., da Silva, S. C., Bittar, C. M., Takiya, C. S., Dorea, J. R. R., del Valle, T. A., Malafaia, P., Santos, F. A. P. 2019. Forestry and Grassland Citrus pulp-based supplement reduces the detrimental effects of high grazing pressure on the performance of beef cattle under a rotational system of Urochloa brizantha Suplemento energético a base de polpa cítrica diminui os efeitos. Rev. Bras. Saúde Prod. Anim. 20:1–14. doi:10.1590/S1519-9940200362019.

Crossland, W. L., Tedeschi, L. O., Callaway, T. R., Miller, M. D.,Smith, W. B. and Cravey, M. 2017. Effects of rotating antibiotic and ionophore feed additives on volatile fatty acid production, potential for methane production, and microbial populations of steers consuming a moderate-forage diet. J. Anim. Sci. 95:4554–4567. doi:10.2527/jas2017.1665.

Donoho, A. L. 1984. Biochemical studies on the fate of monensin in animals and in the environment. J. Anim. Sci. 58:1528–1539. doi:10.2527/jas1984.5861528x.

Dorea, J. R. R., Gouvea, V. N., Agostinho Neto, L. R. D., da Silva, S. C., Brink, G. E., Pires, A. V., Santos, F. A. P. Beef cattle responses to pre-grazing sward height and low level of energy supplementation on tropical pastures. J. Anim. Sci. skaa 163. doi:https://doi.org/10.1093/jas/skaa163.

Drescher, M., I. M. A. Heitkönig, P. J. Van Den Brink, and H. H. T. Prins. 2006. Effects of sward structure on herbivore foraging behaviour in a South African savanna: An investigation of the forage maturation hypothesis. Austral Ecol. 31:76–87. doi:10.1111/j.1442-9993.2006.01552.x.

Edwards, J. E., N. R. McEwan, N. McKain, N. Walker, and R. J. Wallace. 2005. Influence of flavomycin on ruminal fermentation and microbial populations in sheep. Microbiology. 151:717–725. doi:10.1099/mic.0.27602-0.

Ellis, J. L., J. Dijkstra, A. Bannink, E. Kebreab, S. E. Hook, S. Archibeque, and J. France. 2012. Quantifying the effect of monensin dose on the rumen volatile fatty acid profile in high-grain-fed beef cattle. J. Anim. Sci. 90:2717–2726. doi:10.2527/jas.2011-3966.

Ellis, W. C. 1978. Determinants of Grazed Forage Intake and Digestibility. J. Dairy Sci. 61:1828–1840. doi:10.3168/jds.S0022-0302(78)83809-0. Available from: http://dx.doi.org/10.3168/jds.S0022-0302(78)83809-0

van Gastelen, S., J. Dijkstra, and A. Bannink. 2019. Are dietary strategies to mitigate enteric methane emission equally effective across dairy cattle, beef cattle, and sheep? J. Dairy Sci. 102:6109–6130. doi:10.3168/jds.2018-15785.

Van Heijenoort, J. 2001. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. Glycobiology. 11:25R-36R. doi:10.1093/glycob/11.3.25r.

Hodgson, J. 1990. Grazing Management: Science into Practice, Longman Scientific and Technical, New York, NY, USA,1990. Sci. Tech. 1990.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). 2017. Censo agro 2017. Available: https://censos.ibge.gov.br/agro/2017/templates/censo_agro/resultadosagro/estabelecimentos.html.

Ipharraguerre, I. R., and J. H. Clark. 2003. Usefulness of ionophores for lactating dairy cows: A review. Anim. Feed Sci. Technol. 106:39–57. doi:10.1016/S0377-8401(03)00065-8.

Klein, G., and C. M. A. P. Franz. 2005. Chapter 9 The farm animal as potential reservoir of antibiotic resistant bacteria in the food chain. Elsevier Ltd. Available from: http://dx.doi.org/10.1016/S1877-1823(09)70042-6

Kozloski,G.V. 2016. Bioquímica dos ruminantes. 3 ed. Santa Maria: ed. da USFM, 216p.

Lopes, J. C. 2011. Nutrient composition and fiber digestibility measurements of tropical forages collected from intensively managed rotational grazing systems. 53–93.

MacRae, J. C., Bruce, L. A. & Yu, F. 1999. MacRae, J. C., Bruce, L. A. & Yu, F. (1999). The effect of Flavomycin on gastrointestinal leucine metabolism and liveweight gain in lambs. S Afr J Anim Sci 29, 243 – 244. S Afr J Anim Sci. 29.

McGuffey, R. K. 2017. A 100-Year Review: Metabolic modifiers in dairy cattle nutrition. J. Dairy Sci. 100:10113–10142. doi:10.3168/jds.2017-12987. Available from: http://dx.doi.org/10.3168/jds.2017-12987

McGuffey, R. K., L. F. Richardson, and J. I. D. Wilkinson. 2001. Ionophores for Dairy Cattle: Current Status and Future Outlook. J. Dairy Sci. 84:E194–E203. doi:10.3168/jds.s0022-0302(01)70218-4. Available from: http://dx.doi.org/10.3168/jds.S0022-0302(01)70218-4

Mertens, D. R. 1994. Regulation of forage intake. Bioenerg. Wild Herbiv. 81–102. doi:10.1201/9781351070218.

Nagaraja, T. G. (1995). lonophores and antibiotics in ruminants. In Biotechnology in Animal Feeds and Animal Feeding, ed. R. J. Wallace and A. Chesson. VCH Publishers, New York, pp. 173-204.

Van Der Merwe, B. J., T. J. Dugmore, and K. P. Walsh. 2001. The effect of flavophospholipol (Flavomycin®) on milk production and milk urea nitrogen concentrations of grazing dairy cows. South African J. Anim. Sci. 31:101–105. doi:10.4314/sajas.v31i2.3835.

Murray, P.J.; Rowe J.B.; Aitchison, E. M. 1990. The effect of bentonite on wool growth, liveweight change and rumen fermentation in sheep. Aust. J. Exp. Agric. 30:39–42.

Nagaraja, T. G., C. J. Newbold, C. J. van Nevel, and D. I. Demeyer. 1997. Manipulation of ruminal fermentation. Rumen Microb. Ecosyst. 523–632. doi:10.1007/978-94-009-1453-7_13.

Nagaraja, T. G., M. B. Taylor, D. L. Harmon, and J. E. Boyer. 1987. In vitro lactic acid inhibition and alterations in volatile fatty acid production by antimicrobial feed additives. J. Anim. Sci. 65:1064–1076. doi:10.2527/jas1987.6541064x.

Olijhoek, D. W., P. Løvendahl, J. Lassen, A. L. F. Hellwing, J. K. Höglund, M. R. Weisbjerg, S. J. Noel, F. McLean, O. Højberg, and P. Lund. 2018. Methane production, rumen fermentation, and diet digestibility of Holstein and Jersey dairy cows being divergent in residual feed intake and fed at 2 forage-to-concentrate ratios. J. Dairy Sci. 101:9926–9940. doi:10.3168/jds.2017-14278. Available from: http://dx.doi.org/10.3168/jds.2017-14278

Owens, F. N., Basalan, M. 2016. Ruminal fermentation. In Rumenology (pp. 63-102). Springer, Cham.

Pressman, B. C. 1976. Biological Applications of Ionophores. Annu. Rev. Biochem. 45:501–530. doi:10.1146/annurev.bi.45.070176.002441.

Rodrigues, P. H. M. Control and Manipulation of Ruminal Fermentation. In Rumenology (pp. 157-188). Springer, Cham.

Russell, J. B., Onodera, R. & Hino, T. 1991. Russell, J. B., Onodera, R. & Hino, T. (1991). Ruminal protein fermentation: new perspectives on previous contradictions. In Physiological Aspects of Digestion and Metabolism in Ruminants, pp. 681 – 697. Edited by T. Tsuda, Y. Sasaki & R. Kawashima. San D. Physiol. Asp. Dig. Metab. Ruminants. 681 – 697.

Russell, J. B., and A. J. Houlihan. 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiol. Rev. 27:65–74. doi:10.1016/S0168-6445(03)00019-6.

Russell, J. B., and H. J. Strobel. 1989. Effect of Ionophores on ruminal fermentation. Appl. Environ. Microbiol. 55:1–6. doi:10.1128/aem.55.1.1-6.1989.

Schären, M., C. Drong, K. Kiri, S. Riede, M. Gardener, U. Meyer, J. Hummel, T. Urich, G. Breves, and S. Dänicke. 2017. Differential effects of monensin and a blend of essential oils on rumen microbiota composition of transition dairy cows. J. Dairy

Sci. 100:2765–2783. doi:10.3168/jds.2016-11994. Available from: http://dx.doi.org/10.3168/jds.2016-11994

Silva, R. O., L. G. Barioni, J. A. J. Hall, M. Folegatti Matsuura, T. Zanett Albertini, F. A. Fernandes, and D. Moran. 2016. Increasing beef production could lower greenhouse gas emissions in Brazil if decoupled from deforestation. Nat. Clim. Chang. 6:493–497. doi:10.1038/nclimate2916.

Da Silva, S. C., F. M. A. Gimenes, D. O. L. Sarmento, A. F. Sbrissia, D. E. Oliveira, A. Hernadez-Garay, and A. V. Pires. 2013. Grazing behaviour, herbage intake and animal performance of beef cattle heifers on marandu palisade grass subjected to intensities of continuous stocking management. J. Agric. Sci. 151:727–739. doi:10.1017/S0021859612000858.

Soares Filho, C. V., L. R. De Andrade Rodrigues, and S. H. Venturoli Perri. 2002. Produção e valor nutritivo de dez gramíneas forrageiras na região Noroeste do Estado de São Paulo. Acta Sci. Agron. 24:1377. doi:10.4025/actasciagron.v24i0.2385.

T.F. Duffield, Merril, J.K., Bagg, R. N. 2012. Productivity and safety in anesthesia [Productivité et sécurité en anesthésie]. J. Anim. Sci. 15:4583–4592. doi:10.2527/jas2011-5018. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-80054693899&doi=10.1016%2Fj.pratan.2011.08.001&partnerID=40&md5=0ac7ef81dfa63310b3550d4ba8701c97

Tedeschi, L. O., D. G. Fox, and T. P. Tylutki. 2003. Potential Environmental Benefits of Ionophores in Ruminant Diets. J. Environ. Qual. 32:1591–1602. doi:10.2134/jeq2003.1591.

Weimer, P. J., D. M. Stevenson, D. R. Mertens, and E. E. Thomas. 2008. Effect of monensin feeding and withdrawal on populations of individual bacterial species in the rumen of lactating dairy cows fed high-starch rations. Appl. Microbiol. Biotechnol. 80:135–145. doi:10.1007/s00253-008-1528-9.

Wilkinson, J.I.D., Kennington, A.S., Ehrenfried, K.M., Moran, J.W., Buck, J. M. 1997. Wilkinson, J.I.D., Kennington, A.S., Ehrenfried, K.M., Moran, J.W., Buck, J.M., 1997. Human food safety with the use of monensin in lactating dairy cows. In: Proceedings of the Symposium on Usefulness of Ionophores in Lactating Dairy Cattle. Ontario Veten. Proc. Symp. Useful. Ionophores Lact. Dairy Cattle. Ontario Vetenary Coll. Guelph, Canada,. 86–93.

WORLD HEALTH ORGANIZATION. (2016). Critically important antimicrobials for human medicine – Ranking of medically important antimicrobials for risk management of antimicrobial resistance due to non-human use. 5th revision.

4. EFFECTS OF LONG-TERM SUPPLEMENTATION WITH NARASIN, SALINOMYCIN, AND FLAVOMYCIN ON PERFORMANCE AND RUMINAL FERMENTATION CHARACTERISTICS OF BOS INDICUS NELLORE CATTLE FED WITH FORAGE-BASED DIETS

4.1. ABSTRACT

The aim of the present study was to evaluate the inclusion of narasin, salinomycin, and flavomycin for 140 days on ruminal fermentation parameters, apparent nutrient digestibility and performance of Nellore cattle offered a forage-based diet. In Exp. 1, thirty-two rumen-cannulated Bos indicus Nellore steers [initial body weight (BW) = 220 ± 12.6 kg] were assigned to individual pens in a randomized complete block design according to their initial shrunk BW. Within blocks, animals were randomly assigned to 1 of 4 treatments: 1) forage-based diet without feed additives (CON; n = 8), 2) CON diet plus 13 ppm of narasin (NAR; n = 8), 3) CON diet plus 20 ppm of salinomycin (SAL; n = 8), or 4) CON diet plus 3 ppm of flavomycin (FLA; n =8). The experimental period lasted 140 d and was divided into 5 periods of 28 d each. The inclusion of feed additives did not impact ($P \geq 0.17$) dry matter intake (DMI), nutrient intake, and apparent nutrients digestibility. Nonetheless, steers fed NAR had lower ($P < 0.01$) ruminal acetate vs. CON, SAL and FLA, whereas acetate was greater ($P = 0.04$) for SAL vs. CON, tended to be greater ($P = 0.09$) for SAL vs. FLA, and similar ($P = 0.68$) among CON vs. FLA. Ruminal propionate was the highest ($P < 0.01$) for steers fed NAR and similar ($P > 0.20$) among CON, SAL and FLA. Consequently, NAR steers had the lowest ($P < 0.01$) Ac:Pr ratio, whereas Ac:Pr was similar ($P > 0.18$) among CON, SAL, and FLA. Total volatile fatty acids (VFA) was greater ($P < 0.04$) for NAR and CON vs. SAL and FLA, but similar ($P > 0.67$) among NAR vs. CON and SAL vs. FLA. In Exp. 2, 164 Nellore bulls (initial

shrunk BW = 299 ± 2.5 kg) were assigned to collective feedlot pens for 140 d in a randomized complete block design. Within blocks (n = 10), animals were randomly assigned to the same treatments used in Exp. 1. Average daily gain (ADG) was greater ($P < 0.01$) in NAR vs. CON, SAL and FLA bulls, and similar ($P > 0.12$) between CON, SAL, and FLA. Bulls fed NAR had greater ($P < 0.02$) DMI (as kg/d or % BW) and final shrunk BW compared with CON, SAL, and FLA, and similar ($P > 0.26$) between CON, SAL, and FLA, whereas feed efficiency (G:F) was not impacted ($P = 0.51$) by any feed additives used herein. Collectively, narasin was the only feed additive that benefited performance and ruminal fermentation of Nellore animals fed a forage-based diet for a long-term.

Key words: digestibility, feed additives, forage, performance, ruminal parameters, *Bos indicus*

List of abbreviation: Ac:Pr, acetate:propionate; AcBut:Pr, acetate butyrate:propionate; ADG, average daily gain; BW, body weight; CI:C, ground citrus pulp:ground corn; DMI, dry matter intake; FDM, fecal dry matter; G:F, feed efficiency; NCDM, nutrient content of the dry matter intake; NCFM, nutrient content of the fecal dry matter; TTAD, total tract apparent digestibility; VFA, volatile fatty acids

4.2. INTRODUCTION

Beef cattle production system relies mainly in forage-based diets as the source of nutrients for meat production. Accordingly, forages represent up to 81% of the feedstuff required for a beef animal during their productive life (Watson et al., 2015). However, high forage diets frequently do not promote a satisfactory nutrient utilization and animal performance due to limited energy intake (de Souza et al., 2017) and forage physical effect limiting rumen fill (Conrad et al., 1964; Clark and Armentano, 1997). Feed additives have been used as an important nutritional tool to enhance productivity and profitability of beef cattle systems by altering rumen microbiome (Weimer et al., 2008; Schären et al., 2017) and fermentation routes, as well as digestibility and nutrient utilization of the diet (Tedeschi et al., 2003). Nonetheless, the majority of research conducted to date within feed additives focused on high-concentrate based diets (Duffield et al., 2012) with addition of monensin or lasalocid as ionophores and little is known about the effects of others feed additives on B. indicus cattle fed high-forage diets. Additionally, it is important to establish if the use of feed additive in forage-based diets for a long-term (Rodgers et al., 1997; Odongo et al., 2007) would impact the persistence of efficacy, once a diminishing response due to rumen microbial adaptation might occur when feed additives are fed over a prolonged period (Klein et al., 2005).

Narasin is an ionophore that alters rumen fermentation dynamics (Miszura et al., 2018), plasma metabolites (Sardinha et al., 2020; Polizel et al., 2020) and improves animal performance (Silva et al., 2015; Polizel et al., 2020). Salinomycin is also an ionophore that improves animal production by favorably altering molar acetate:propionate ratio (McClure et al. 1980; Bagley et al., 1988). Flavomycin (bambermycin) is a non-ionophore antibiotic that prevents synthesis of peptidoglycan on the bacterial cell wall (Volke et al., 1997) and might have indirect benefits on gut tissue

protein turnover by also suppressing gram-negative pathogenic bacteria (Edwards et al., 2005), as well as gram-positive bacteria which might allow increased dietary fermentation, resulting in a greater propionate proportion (Edrington et al., 2003). Although feed additives have similar ruminal modes of action, animal performance might vary depending on dosage, animal, and diet (Bretschneider et al., 2008). Based on this rationale, we hypothesized that long-term supplementation with narasin, salinomycin, or flavomycin would impact nutrient digestibility, change rumen fermentation parameters, and improve productivity of *B. indicus* Nellore cattle fed a forage-based diet. To test this hypothesis, the objective of this experiment was to evaluate the impacts of supplementing narasin, salinomycin, or flavomycin on rumen fermentation characteristics and apparent nutrient digestibility (Exp. 1), as well as feed intake, and growth (Exp. 2) of B. indicus cattle fed a high-forage diet for 140 days.

4.3. MATERIALS AND METHODS

These studies were conducted at the University of São Paulo, Piracicaba campus (USP/ESALQ; Piracicaba, SP, Brazil; 22º43’31’’ S, 47º38’51’’W, and 524 m elevation). 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 São Paulo; CEUA/FMVZ; protocol #8582080119).

4.3.1. Experiment 1 – Animal Metabolism

Animals, Housing and Diets

Thirty-two rumen-cannulated *B. indicus* Nellore steers [initial body weight (**BW**) = 220 ± 12.6 kg; age = 20 ± 1.0 mo] were assigned to individual pens (concrete-surface; 2 × 2 m, with a feed bunk and waterer) in a randomized complete block design according

to their initial shrunk BW. Within blocks (n = 8), animals were randomly assigned to 1 of 4 treatments: **1)** forage-based diet without feed additives (**CON**; *n* = 8), **2)** CON diet plus 13 ppm of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil; **NAR**; *n* = 8), **3)** CON diet plus 20 ppm of salinomycin (Posistac; Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil; **SAL**; *n* = 8), or **4)** CON diet plus 3 ppm of flavomycin (Flavomycin 80, Huvepharma, Porto Alegre, Rio Grande do Sul, Brazil; **FLA**; *n* =8). The administration rates of NAR, SAL, and FLA used herein were according to manufacturer's recommendation. The experimental period lasted 140 d and was divided into 5 periods of 28 d each (0, 28, 56, 84, 112 and 140 d).

Throughout the experimental period (d 0 to 140), steers were offered Tifton-85 haylage (*Cynodon dactylon* spp.) which was chopped daily with a vertical mixer (Mixer VM8B, DeLaval International AB, Tumba, Sweden). Haylage average particle length distribution was 50.3 ± 2.5% > 19 mm; 25.8 ± 3.2% > 8 mm; 16.1 ± 1.8 % > 4 mm; and 7.8 ± 2.0% on bottom sieve according to Penn State Particle Separator procedures (Heinrichs, 1996; Kononoff et al., 2003). Feed additives (NAR, SAL, and FLA) were separately mixed with a 50:50 mixture of ground citrus pulp:ground corn (**CI:C**; 25 g of each ingredient used as a delivery vehicle; as-fed basis) . The initial inclusion of feed additives treatment in the 50:50 CI:C mixture was based on a 5.0 kg of forage DMI. Hence, for steers consuming 5.0 kg of forage, the CI:C mixture would contain 65, 100 and 15 ppm of narasin, salinomycin and flavomycin for NAR, SAL and FLA, respectively. Steers from CON group also received the CI:C supplement without the inclusion of feed additives.

Treatments (NAR, SAL, FLA, and CON) were offered to each pen individually and daily prior to haylage feeding to avoid that small amount of supplement would be mixed with hay and compromise intake of the feed additives treatments. Treatments

amount were calculated based on the previous day individual total forage dry matter intake (**DMI**). From d 0 to 140, animals were fed treatments once daily (0800 h) and had ad libitum access to haylage (0830 h), mineral-mixed (offered in separately feed bunk from the haylage and treatments), and fresh water. Steers promptly consumed treatments within 30 min after feeding. The mineral mix (Premiphós 80; Premix; Ribeirão Preto, SP, Brazil) used herein contained 150 g/kg Ca, 80 g/kg P, 12 g/kg S, 134 g/kg Na, 4,500 mg/kg Zn 1,600 mg/kg, 1,400 mg/kg Mn, 800 mg/kg F, 210 mg/kg Co, 180 mg/kg I, and 27 mg/kg Se. The nutritional profile of the haylage and supplement used herein is described in Table 2.

Sampling, laboratory analyses, and measurements.

Samples of haylage and CI:C supplement were collected weekly, pooled across all weeks within each period, and analyzed for nutrient profile (Table 2). From d 23 to 27 (period 1), 51 to 55 (period 2), 79 to 83 (period 3), 107 to 111 (period 4), and 135 to 139 (period 5), total fecal production was individually collected to determine apparent nutrient digestibility. Total fecal production was collected and quantified twice a day using an electronic scale (Marte AC-10K; Marte Cientifica, São Paulo, SP, Brazil) at 0800 h and 1800 h, and a representative sample (approximately 10% of wet weight) of the daily production of each steer was collected and stored at -18°C on the same day of collection. Apparent nutrient digestibility was calculated according to the formula: TTAD (%) = ((DMI × NCDM) – (FDM × NCFM) × 100) / (DMI × NCDM), where TTAD = total tract apparent digestibility, DMI = dry matter intake, NCDM = nutrient content of the DMI (%), FDM = fecal dry matter, and NCFM = nutrient content of the fecal DM (%).

Samples of feed, orts, and feces were dried in a forced-air oven at 60°C (AOAC, 1990; method #930.15) for 96 h. 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 for 24 h (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 neutral detergent fiber (**NDF;** Van Soest et al., 1991) and acid detergent fiber (**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 extract ether content was determined using an Ankom^{XT15} Extrator (Ankom Technology, Macedon, USA), according to AOAC (1990; method 920.29), using petroleum ether. 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. Calculation of haylage and supplement total digestible nutrients (**TDN**), net energy for maintenance ($\mathbf{NE_m}$) and gain ($\mathbf{NE_g}$) was performed according to Weiss et al. (1992) and the tabular values proposed by NASEM (2016).

Individual shrunk BW was collected on d 0 after 14 h of feed and water withdrawal to determine initial BW and to perform the randomization into blocks and treatments. Forage, supplement, and total DMI were recorded daily from each pen by collecting and weighing non-consumed feed (forage only). Samples of the offered and non-consumed feed were collected daily from each pen and dried for 24 h at 105 ± 2 °C in forced-air ovens for dry matter calculation.

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 CI:C supplement feeding, ruminal fluid samples were manually collected (approximately 100 mL) by squeezing the ruminal contents into 4 layers of cheesecloth and the ruminal fluid pH was immediately determined (Digimed-M20; Digimed

Instrumentação Analítica; São Paulo, SP, Brazil). Approximately 50 mL of the ruminal fluid were collected and stored at -18°C for subsequent analysis of rumen ammonia and molar proportions of individual volatile fatty acids (**VFA**; acetate, propionate, butyrate, isobutyrate, valerate, isovalerate), as well as the acetate:propionate (**Ac:Pr**) and acetate butyrate:propionate (**AcBut:Pr**) ratios, and total VFA. Frozen ruminal samples were prepared for analysis by thawing, centrifuging (15,000 × g) for 60 min at 4ºC, and analyzed for VFA and rumen ammonia according to procedures described by Ferreira et al. (2016) and Broderick and Kang (1980), respectively.

4.3.2. Experiment 2 – Animal Performance

Animals, Housing, and Experimental Design

One hundred and sixty-four *B. indicus* Nellore bulls (initial shrunk BW = 298.95 ± 2.5; age = 23 ± 3.0 mo) were assigned to collective pens in a randomized complete block design according to their shrunk BW (after 14 h of feed and water restriction). 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 to 5 bulls per pen; 18 m²) with a concrete floor, feed bunk, mineral bunk, and waterer. Within blocks (n = 10), animals were randomly assigned to the same treatments as in Experiment 1.

Throughout the experimental period (d 0 to 140), bulls were offered Tifton-85 haylage (*Cynodon dactylon* spp.) which was chopped daily utilizing a vertical mixer (Mixer VM8B, DeLaval International AB). Haylage average particle length distribution was 46.7 ± 3.1 % > 19 mm, 28.1 ± 2.1% > 8 mm, 15.2 ± 2.0 % > 4 mm, and 10 ± 3.8% on bottom sieve according to Penn State Particle Separator procedures (Heinrichs, 1996; Kononoff et al., 2003), whereas ground corn was used as a delivery vehicle for feed additives treatments (NAR, SAL, and FLA). Additionally, animals from CON group also received ground corn with no inclusion of feed additives. Feed additives were mixed into

ground corn (200 g/pen for each 5 kg of haylage DMI; as-fed basis) and offered to each pen individually. Bulls promptly consumed the supplement within 30 min after feeding and then the haylage was offered. Treatments were offered daily prior to haylage feeding to avoid that the small amount of concentrate would be mixed with the hay and compromise the intake of feed additives. The nutritional profile of the forage used in the present experiment is described in Table 3.

From d 0 to 140, animals were fed the treatments (ground corn with or without feed additives) once daily at 0730 h and had ad libitum access to haylage (0800 h), mineral-vitamin mix, and fresh water. Mineral mix (Premiphós 80; Premix) used herein was the same as in Exp. 1 and was offered separately in feed bunk from haylage and treatments. The initial inclusion of additives in the ground corn was based on a 5.0 kg of forage DMI. Hence, for animals consuming 5.0 kg of forage, the ground corn would contain 65, 100 and 15 ppm of narasin, salinomycin and flavomycin, for NAR, SAL, and FLA, respectively. The doses of NAR, SAL, and FLA used herein were according to manufacturer's recommendation. Throughout the experimental period (d 0 to 140), additives dosage offered to the animals was based on the previous day total DMI.

Sampling and Measurements

At the beginning (d 0) of the experimental period, individual shrunk BW was recorded after 14 h of feed and water withdrawal to determine animal initial BW and to perform the randomization of the animals into blocks and treatments. To calculate average daily gain (**ADG**) and feed efficiency (**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. Dry matter intake was evaluated daily from each pen within each period by collecting and weighing non-consumed feed weekly. Hay and total DMI of each pen were divided by the number of bulls within each pen and expressed as kg per bull/day. Within each pen,

total BW gain and total DMI of each period were used for bull G:F calculation. Samples of feed and orts were collected weekly, pooled across all weeks within each period, and analyzed for nutrient profile as aforementioned for Exp.1.

Statistical analyses

For all the variables analyzed, animal (Exp. 1) or pen (Exp. 2) were considered the experimental unit and quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.; Cary, NC). All data were analyzed using Satterthwaite approximation to determine the denominator df for the test of fixed effects, with animal(treatment) for Exp. 1 or animal(pen) and pen(treatment) for Exp. 2 as random variables. Model statement for all analyzes contained the effects of treatment, day or period, and treatment × day or period interactions and block as independent covariate. The specified term for all repeated statements was day or period, with animal(treatment) as subject for Exp. 1, and pen(treatment) as subject for DMI and G:F only, and animal(pen) as subject for all other analyses for Exp. 2. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables analyzed. All results from Exp. 1 are reported as covariately-adjusted least square means for values obtained on d 0, except for forage DMI, and separated using PDIFF. All results from Exp. 2 are reported as least square means and were separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Repeated measures are reported according to main treatment effect if the treatment × day interaction was $P > 0.10$.

4.4. RESULTS

Experiment 1 - Animal Metabolism

Values obtained on day 0 of the study were not significant covariates ($P > 0.56$) for rumen concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate, and did not differ among treatments ($P > 0.28$; data not shown), demonstrating that animals were under a similar management prior to the beginning of the present study.

No treatment × period interactions were identified for intake and apparent nutrient digestibility ($P \geq 0.33$; Table 4) for steers receiving the experimental treatments. The inclusion of feed additives did not impact (main treatment effect; $P \geq 0.17$) DMI and specific nutrient intake (Table 4). In addition, there was no effect ($P \geq 0.40$) on apparent nutrient digestibility among treatments (Table 4). However, there was a period effect ($P < 0.001$) on intake and nutrient digestibility ($P < 0.01$; Table 4), which may be attributed to the variation observed on the quality and composition of forage during the experiment period (Table 2).

A treatment × day interaction was only detected ($P < 0.01$) for AcBut:Pr ratio. After the second experimental period, animals fed NAR had the smallest values for AcBut:Pr ratio, whereas it was similar ($P > 0.28$) among SAL, FLA, and CON. A treatment effect was detected ($P \leq 0.02$) for molar concentration of acetate, propionate, butyrate, isovalerate, as well as Ac:Pr, and AcBut:Pr ratios. In general, animals fed NAR had the lowest ($P < 0.01$) ruminal molar concentration of acetate compared with CON, SAL and FLA, whereas acetate concentration was greater ($P = 0.04$) for SAL vs. CON, tended to be greater ($P = 0.09$) for SAL vs. FLA, and similar ($P = 0.68$) among CON vs. FLA (Table 5). On the other hand, the molar concentration of propionate was highest ($P < 0.01$; Table 5) for animals fed NAR and similar ($P > 0.20$) among CON, SAL and FLA.

Consequently, NAR animals had the lowest ($P < 0.01$) Ac:Pr ratio, whereas Ac:Pr ratio was similar ($P > 0.18$) among CON, SAL, and FLA (Table 5).

A treatment effect was detected ($P < 0.01$) for molar concentration of butyrate, which was reduced ($P < 0.01$) for animals fed NAR compared with CON and FLA, whereas butyrate concentration was similar ($P > 0.36$) among NAR vs. SAL, CON vs. FLA, and tended to be lower ($P = 0.09$) for SAL vs. FLA. A treatment effect was also detected ($P < 0.01$) for total VFA, which was greater ($P < 0.04$) for NAR and CON compared with SAL and FLA, but similar ($P > 0.67$) among NAR vs. CON and SAL vs. FLA (Table 5). No treatment effect was detected ($P > 0.11$) for isobutyrate, isovalerate, valerate, ruminal ammonia, and pH (Table 5). A day effect was observed ($P < 0.01$) for all rumen variables herein analyzed (Table 5).

Experiment 2 – Animal Performance

As designed, initial BW was similar ($P = 0.99$) among treatments (Table 6). During experiment, ADG was greater ($P < 0.01$) in NAR vs. CON, SAL, and FLA bulls, and similar ($P > 0.12$) between CON, SAL, and FLA (Table 6; main treatment effect, $P < 0.01$). A treatment × period interaction was detected ($P = 0.03$) for DMI, which was greater ($P < 0.01$) for NAR bulls on period 2, 4, and 5 of the experiment compared with CON, SAL, and FLA, and similar ($P > 0.26$) between CON, SAL, and FLA (Table 6; Fig. 3). A tendency was detected ($P = 0.08$; treatment × period interaction) for DMI as % BW, which was also greater ($P < 0.01$) for NAR bulls compared with CON, SAL, and FLA, and similar ($P > 0.26$) between CON, SAL, and FLA (Table 6). No treatment effect was detected ($P = 0.51$) for G:F, whereas final shrunk BW was greater ($P = 0.02$, main treatment effect) for NAR animals compared with CON, SAL, and FLA, and similar ($P > 0.52$) between CON, SAL, and FLA (Table 6).

4.5. DISCUSSION

Feed additives have been used as an important management tool to enhance cattle growth and feed efficiency by altering ruminal fermentative routes, digestibility, and nutrient utilization of the diet (Tedeschi et al., 2003; Duffield et al., 2012). Nonetheless, the majority of research conducted to date with feed additives focused on high-concentrate based diets (Tedeschi et al., 2003; Duffield et al., 2008; 2012; Ellis et al., 2012) and with monensin or lasalocid as the ionophore, whereas little is known about the effects of others feed additives (ionophore or non-ionophore) on *B. indicus* Nellore cattle fed high-forage based diets. Moreover, there are limited or inconsistent information about the impacts of feed additives on DMI of forage-based diets fed for a long-term (Bretschneider et al., 2008). Given the limited body of research investigating the efficacy and long-term inclusion of ionophores (narasin and salinomycin) or non-ionophore (flavomycin) on ruminal fermentative parameters and performance of Nellore cattle fed high forage-based diets, results from this experiment are also being contrasted with studies using others feed additives and *Bos taurus* cattle.

It is known that the inclusion of ionophores into beef cattle diets alters ruminal fermentation dynamics by changing microbial ecosystem favoring microorganisms, mostly bacteria Gram-negative that are insensitive to the action of ionophores (Tedeschi et al., 2003; Duffield et al., 2012). Most of ionophores (lasalocid, monensin, salinomycin, laidlomycin, and narasin) in the market are produced by *Streptomyces spp*. (Nagaraja, 1995) and their mechanisms are similar in the rumen, whereas animal performance might vary depending on dosage, animal, and diet (Nagaraja et al., 1987; Tedeschi et al., 2003; Bretschneider et al., 2008). Narasin, an ionophore used in this study, is produced by the *Streptomyces aureofaciens* and also changes the fermentation dynamics in the rumen toward increased propionate and decreased acetate by impacting gram-positive bacteria

on animals fed with high-forage diets (Miszura et al., 2018; Polizel et al., 2020). Salinomycin is also an ionophore produced by *Streptomyces albus*, which has been shown to improve animal production on pasture (McClure et al., 1980) by altering favorably molar ration of acetate and propionate (Bagley et al., 1988), whereas results of forage-fed beef cattle have been inconsistent. Flavomycin (bambermycin) is a non-ionophore antibiotic produced by *Streptomyces bambergiensis, S. geysirensis,* and *S. ederensis*, that prevents synthesis of peptidoglycan on the bacterial cell wall (Volke et al., 1997) and might have indirect benefits on gut tissue protein turnover by also suppressing Gram-negative pathogenic bacteria, such as *Fusobacterium spp*. (Edwards et al., 2005), as well as Gram-positive bacteria which might allow increased dietary fermentation, resulting in a greater propionate proportion (Edrington et al., 2003). Moreover, flavomycin may be capable of altering ruminal protozoa population, which in turn might improve fiber digestion (Perry, 2002), and performance of forage-based livestock systems (Beck et al., 2016).

It is still controversy in the literature the impacts of ionophores and non-ionophores on nutrient digestibility (Wedegaertner and Johnson, 1983; Ricke et al., 1984; Crossland et al., 2017; Polizel et al., 2020). In the current study, the inclusion of feed additives into forage-based diets did not impact apparent digestibility of nutrients (Exp.1). In agreement with our data, Bell et al. (2017) reported no differences in nutrient digestibility of beef steers receiving a forage-based diet with or without monensin. Accordingly, Polizel et al. (2020) also observed no differences on apparent digestibility of nutrients of *B. indicus* Nellore steers receiving a high forage-based diets with addition or not of narasin. Corroborating with our results, Kobayashi et al. (1992) reported no differences in apparent digestibility of nutrients in wethers supplemented with salinomycin. Crossland et al. (2017), however, observed that flavomycin or monensin

supplementation decreased dry matter digestibility from week 1 to 3 and increased at week 6 in steers consuming a moderate-forage based diet. The authors attributed these findings to a short-term inefficiency in fermentation parameters of flavomycin and monensin as the microbial population are adjusting.

Feed additives impact ADG, G:F, and DMI of animals offered a high-concentrate diet (Duffield et al., 2012; Golder and Lean, 2016). Similar results were observed, except for DMI, in animals offered a high-forage diet (Bretschneider et al., 2008). In the currently study (Exp. 2 only), intake was 7.9, 8.8, and 10.7 % greater for animals offered NAR compared with CON, FLA, and SAL, respectively. Similar results were observed when intake was expressed as % of BW. Corroborating with our results, Miszura et al. (2019) observed that animals receiving narasin increased DMI by 7.55% in high forage-based diets. Conversely, studies reported that inclusion of narasin did not impact forage DMI in animals offered a high forage-based diets (Silva et al., 2015; Polizel et al., 2016; Pascoalino et al., 2020). Bretschneider et al. (2008) reported that inclusion of ionophores in diets with high inclusion of forage did not impact DMI. The effect of ionophores on DMI might depend on the forage quality consumed by the animals which can impact passage rate and gut fill, and consequently DMI response (Ellis et al. 1984). Nevertheless, the impact of ionophores and non-ionophores on DMI of beef cattle consumed high forage-based diets deserves further investigation. Also, research is warranted to understand the effects of feed additive supplementation on beef cattle fed high-forage based diets.

Inclusion of feed additives in beef cattle diets normally impact feed efficiency by improving or maintaining ADG and reducing DMI (Tedeschi et al., 2003: Bretschneider et al., 2008 Duffield et al., 2012). In the current study, only narasin improved ADG by 14.8, 11.8, and 7.8% compared with CON, SAL, and FLA, respectively, which resulted

in heavier animals at the end of the supplementation period. These outcomes are partially resultant from difference in ruminal fermentation parameters in animals supplemented with narasin, given that increasing molar concentration of propionate and total VFA, and decreasing acetate and butyrate in the rumen are positively correlated with greater feed energy utilization and performance (Blaxter, 1962; Russel and Strobel, 1989; McGuffey et al., 2001; Weimer, et al., 2008). Supporting our results, others have also reported increased concentration of rumen propionate and total VFA, and reduced concentration of rumen acetate and butyrate when narasin was fed to beef cattle (Miszura et al., 2018; Polizel et al., 2020). Also corroborating with this study, flavomycin (Mogentale et al., 2010; Crossland et al., 2017) or salinomycin (Olumeyan et al., 1986; Zinn, 1986) supplementation to beef cattle consuming or not a forage-based diets was not capable of changing ruminal fermentation parameters. In fact, Olumeyan et al. (1986) and Zinn (1986) reported that rumen fermentation only changed when the diets had increased amount of grain. Despite the difference in BW, DMI, and ruminal fermentation parameters herein, they were not sufficient to influence feed efficiency of Nellore bulls consuming a high forage-based diet with the addition of ionophores or non-ionophores.

Similar ruminal pH values were expected, given that all animals consumed a forage-based diets and only a small amount of grain were used as a delivery vehicle for the feed additives. Therefore, it is likely that ruminal pH values were maintained in a range that would not impair rumen and cellulolytic bacteria function. Supporting this statement, Bell et al (2017) and Polizel et al. (2020) also reported similar rumen pH values of beef steers offered a high forage-based diets with monensin and narasin, respectively. Accordingly, Crossland et al. (2017) did not observe any effect of flavomycin or monensin supplementation on rumen pH of beef steers offered a moderate forage-based

diet. In fact, the ruminal pH in the present study was within a range that supports and maintains adequate fiber digestion in ruminants (Yokoyama and Johnson, 1988).

Feed additives might mitigate ruminal proteolysis and subsequently reduce ammonia synthesis (Goodrich et al., 1984; Rogers et al., 1997). Moreover, rumen ammonia levels below 5 mg/dL might limit microbial growth and ruminal fermentation parameters (Satter and Slyter, 1974; Slyter et al., 1979). Feed additives used herein were not capable of impacting ruminal ammonia concentration of beef steers offered a high forage-based diet, despite the permanent impact on ruminal VFA profile. Supporting our data, Bell et al. (2017) demonstrated that long-term supplementation with monensin did not impact rumen ammonia concentration of beef steers fed a high forage-based diet. Similarly, Lemos et al. (2016) reported no difference in rumen ammonia concentration of beef cattle fed a concentrate diet with flavomycin or monensin.

One of the hypotheses of the present study was that feed additives might not have a long-term effect (Guan et al., 2006) and that rumen microbiome adapts to these feed additives (Crossland et al., 2017). Nonetheless, our data demonstrated that only narasin had a long-term impact on rumen fermentation parameters (Fig. 2). Accordingly, Polizel et al. (2020) observed a long-term effect of narasin on ruminal parameters of beef cattle fed with high forage-based diets for 140 d. Despite the differences in rumen fermentation, animals fed diet containing narasin had higher and persistent DMI (Fig. 4), resulting in heavier animals (Fig. 3) at the end of the experimental period. Nevertheless, studies are warranted to further understand the benefits of narasin supplementation for an extend period in beef cattle consuming forage-based diets.

Collectively, inclusion of feed additives (ionophore and non-ionophore) in high forage-based diet did not impact nutrient intake and apparent digestibility of nutrients. Conversely, only narasin was able to fully alter rumen VFA profile by impacting the

molar concentration of acetate, butyrate, propionate, and total VFA in Nellore steers fed high forage-based diet for an140-d period. These outcomes might, at least partially, contribute to the improved ADG and final BW of Nellore bulls supplemented with narasin, despite the concurrent increase in DMI. Nonetheless, results from this experiment suggest that supplementing narasin to *B. indicus* Nellore cattle for 140 days might be a feasible alternative to optimize rumen fermentation characteristics and productivity in grazing beef cattle.

4.6. DISCLOSURES

No conflict of interest to disclose.

4.7. ACKNOWLEDGMENT

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

4.8. LITERATURE CITED

Association of Official Analytical Chemists (AOAC). 1990. Official methods of analysis. 12th ed. AOAC, Washington, DC.

Bagley, C. P., J. I. Feazel, D. G. Morrison, and D. M. Lucas. 1988. Effects of salinomycin on ruminal characteristics and performance of grazing beef steers 1 I ntroduetion Bermudagrass (Cynadon dactylon) is a. 792–797.

Beck, P., W. Galyen, D. Galloway, E. B. Kegley, R. Rorie, D. Hubbell, J. Tucker, T. Hess, M. Cravey, J. Hill, et al. 2016. Effect of supplementation of developing replacement heifers with monensin or bambermycins on gain and pregnancy rates. The Professional Animal Scientist 32(5):619–626. doi: 10.15232/pas.2016-01525

Bell, N. L., R. C. Anderson, T. R. Callaway, M. O. Franco, J. E. Sawyer, and T. A. Wickersham. 2017. Effect of monensin inclusion on intake, digestion, and ruminal fermentation parameters by *Bos taurus indicus* and *Bos taurus taurus* steers consuming bermudagrass hay. J. Anim. Sci. 95:2736–2746. doi: 10.2527/jas.2016.1011

Blaxter, K.L. 1962. Energy Metabolism of Ruminants. Charles C. Thomas, Springfield, IL.

Bretschneider, G., J. C. Elizalde, and F. A. Pérez. 2008. The effect of feeding antibiotic growth promoters on the performance of beef cattle consuming forage-based diets: A review. Livest. Sci. 114:135–149. doi:10.1016/j.livsci.2007.12.017.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64–75. doi:10.3168/jds.S0022-0302(80)82888-8

Clark, P. W.; L. E. Armentano, 1997. Influence of particle size on the effectiveness of

beet pulp fiber. J. Dairy Sci. 80:898–904. doi:10.3168/jds.S0022-0302(97)75986-1

Conrad, H.R., A. D. Pratt, and J. W. Hibbs. 1964. Regulation of feed intake in dairy cows. I. Change in importance of physical and physiological factors with increasing digestibility. J. Dairy Sci. 47:54-62. doi:10.3168/jds.S0022-0302(64)88581-7.

Crossland, W. L., L. O. Tedeschi, T. R. Callaway, M. D. Miller, W. B. Smith, and M. Cravey. 2017. Effects of rotating antibiotic and ionophore feed additives on volatile fatty acid production, potential for methane production, and microbial populations of steers consuming a moderate-forage diet. J. Anim. Sci. 95:4554–4567. doi:10.2527/jas2017.1665.

de Souza, J., F. Batistel, and F.A.P. Santos. 2017. Effect of sources of Ca salts of fatty acids on production, nutrient digestibility, energy balance, and carryover effects of early lactation grazing dairy cows. J. Dairy Sci. 100:1072–1085. doi:10.3168/jds.2016-11636.

Duffield, T. F., A. R. Rabiee, and I. J. Lean. 2008. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 1. Metabolic effects. J. Dairy Sci. 91:1334–1346. doi:10.3168/jds.2007-0607

Duffield, T. F., J. K. Merrill, and R. N. Bagg. 2012. Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain and dry matter intake. J. Anim. Sci. 90: 4583-4592. doi: 10.2527/jas.2011-5018.

Edrington, T. S., T. R. Callaway, P. D. Varey, Y. S. Jung, K. M. Bischoff, R. O. Elder, R. C. Anderson, E. Kutter, A. D. Brabban, and D. J. Nisbet. 2003. Effects of the antibiotic ionophores monensin, lasalocid, laidlomycin propionate and

bambermycin on Salmonella and E. coli O157:H7 in vitro. J. Appl. Microbiol. 94(2):207–213. doi:10.1046/j.1365-2672.2003.01822.x.

Edwards, J. E., B. J. Bequette, N. McKain, N. R. McEwan, and R. J. Wallace. 2005a. Influence of flavomycin on microbial numbers, microbial metabolism and gut tissue protein turnover in the digestive tract of sheep. Br. J. Nutr. 94:64–70. doi:10.1079/ BJN20051444.

Ellis, J. L., J. Dijkstra, A. Bannink, E. Kebreab, E. Hook, S. Archibeque, and J. France. 2012. Quantifying the effect of monensin dose on the rumen volatile fatty acid profile in high-grain-fed beef cattle. J. Anim. Sci. 90:2717-2726. doi: 10.2527/jas.2011-3966.

Ellis, W. C., G. W. Horn, D. Delaney, and K. R. Pond. 1984. Effects of ionophores on grazed forage utilization and their economic value for cattle on wheat pasture. In: G.W. Horn, editor. Proc. Nat. Wheat Pasture Symp. Stillwater, OK: Oklahoma State University, pp. 343–355. MP-115.

Ferreira, E. M., A. V. Pires, I. Susin, M. V. Biehl, R. S. Gentil, M. O. M. Parente, D. M. Polizel, C. V. D. M. Ribeiro, and E. Almeida. 2016. Nutrient digestibility and ruminal fatty acid metabolism in lambs supplemented with soybean oil partially replaced by fish oil blend. Anim. Feed Sci. Technol. 216:30-39. doi: 10.1016/j.anifeedsci.2015.09.007

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus reagents, and some applications). United States Department of Agriculture, Washington, D.C.

Golder, H. M., and I. J. Lean. 2016. A meta-analysis of lasalocid effects on rumen measures, beef and dairy performance, and carcass traits in cattle1. J. Anim. Sci. 94:306–326. doi:10.2527/jas.2015-9694.

Goodrich, R. D., J. E. Garrett, D. R. Gast, M. A. Kirick, D. A. Larson, and J. C. Meiske. 1984. Influence of monensin on the performance of cattle. J. Anim. Sci. 58:1484–1498. doi:10.2527/jas1984.5861484x

Guan, H., K. M. Wittenberg, K. H. Ominski, and D. O. Krause. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. J. Anim. Sci. 84:1896–1906. doi:10.2527/jas.2005-652

Heinrichs, J., P. Kononoff. 1996. Evaluating particle size of forages and TMRs using the Penn State Particle Size Separator. The Pennsylvania State University, University Park, PA, 1-14.

Klein, G., and C. M. A. P. Franz. 2005. Chapter 9 The farm animal as potential reservoir of antibiotic resistant bacteria in the food chain. Elsevier Ltd. doi: 10.1016/S1877-1823(09)70042-6

Kobayashi, Y., M. Wakita, S. Hoshino, S. 1992. Effects of ionophore salinomycin on nitrogen and long-chain fatty acid profiles of digesta in the rumen and the duodenum of sheep. Anim. Feed Sci. Technol. 36, 67–76.

Kononoff, P.J., A. J. Heinrichs, D.R. Buckmaster. 2003.Modification of the penn state forage and total mixed ration particle separator and the effects of moisture content on its measurements. J. Dairy Sci. 86:1858-1863. doi:10.3168/jds.S0022-0302(03)73773-4

Lemos B. J. M., F. G. F. Castro, L. S. Santos, B. P. C. Mendonça, V. R. M. Couto, J. J. R. Fernandes. 2016. Monensin, virginiamycin, and flavomycin in a no-roughage finishing diet fed to zebu cattle. *J. Anim. Sci.* 94:4307–4314. doi.org/10.2527/jas.2016-0504

McClure, W. P., J. P. Fontenot, K. E. Webb, Jr. and D. M. Lucas. 1980. Feedlot performance of cattle fed different salinomycin levels. J. Anim. Sci. 51(Suppl. 1): 380.

McGuffey, R. K., L. F. Richardson, and J. I. D. Wilkinson. 2001. Ionophores for Dairy Cattle: Current Status and Future Outlook. J. Dairy Sci. 84:E194–E203. doi:10.3168/jds.s0022-0302(01)70218-4.

Miszura, A. A., D. M. Polizel, J. P. R. Barroso, A. S. Martins, G. B. Oliveira, A. C. Limede, L. A. Sardinha, F. M. Neto, R. S. Marques, A. V. Pires. 2019. Effects of feed additives on performance of yearling bulls fed high forage diet. J. Anim. Sci. 97 (E-Suppl. 3):425. doi.org/10.1093/jas/skz258.843

Miszura, A. A., D. M. Polizel, M. Ferraz Jr., J. Barroso, L. Gobato, A. Martins, G. Oliveira, E. Ferreira, A. Pires. 2018. Effects of feed additives on rumen parameters of steers fed a high-forage diet. J. Anim. Sci. 96 (E-Suppl. 3):441. doi.org/10.1093/jas/sky404.964

Mogentale S. M., E. J. A. Silva, P. M. Meyer, C. T. Marino, M. C. A. Sucupira, J. J. A. A. Demarchi and P. H. M. Rodrigues. 2010. Effects of Flavomycin on Ruminal Fermentation, *In Situ* Degradability and *In Vivo* Digestibility in Bovine Fed Sugarcane Diets. Am. J. Anim. Vet. Sci. 5: 76-85. **doi:**10.3844/ajavsp.2010.76.85

Nagaraja, T. G., M. B. Taylor, D. L. Harmon, and J. E. Boyer. 1987. In vitro lactic acid inhibition and alterations in volatile fatty acid production by antimicrobial feed additives. J. Anim. Sci. 65:1064–1076. doi: 10.2527/jas1987.6541064x

Nagaraja, T.G. 1995. Ionophores and antibiotics in ruminants. p. 173– 204. In R.J.Wallace and A. Chesson (ed.) Biotechnology in animal feeds and animal feeding. VCH Publ., New York.

NASEM. 2016. Nutrient requirements of beef cattle. 8th ed. Washington, DC: The National Academics Press

Odongo, N. E., R. Bagg, G. Vessie, P. Dick, M. M. Or-Rashid, S. E. Hook, J. T. Gray, E. Kebreab, J. France, and B. W. McBride. 2007. Long-term effects of feeding monensin on methane production in lactating dairy cows. J. Dairy Sci. 90:1781–1788. doi:10.3168/jds.2006-708.

Olumeyan, D. B., T. G. Nagaraja, G. W. Miller, R. A. Frey, and J. E. Boyer. 1986. Rumen microbial changes in cattle fed diets with or without salinomycin. Appl. Environ. Microbiol. 51:340–345. doi:10.1128/aem.51.2.340-345.1986.

Pascoalino, L. F., G. B. Oliveira, A. A. Miszura, J. P. R. Barroso, A. C. Limede, L. A. Sardinha, J. S. Biava, E. M. Ferreira, A. V. Pires, D. M. Polizel. 2020. Residual effect of narasin on ruminal fermentation characteristics in lambs. Livestock Science. 240, 104141. doi: 10.1016/j.livsci.2020.104141

Perry, T. W. 2002. Feeds and Feed Additives, Ruminant Feeds. In: Kirk-Othmer Encyclopedia of Chemical Technology, (Ed.) doi: 10.1002/0471238961.1821130919211422.a01.pub2.

Polizel, D. M., B. I. Cappellozza, F. Hoe, C. N. Lopes, J. P. Barroso, A. Miszura, G. B. Oliveira, L. Gobato, and A. V. Pires. 2020. Effects of narasin supplementation on dry matter intake and rumen fermentation characteristics of *Bos Indicus* steers fed a high-forage diet. Transl. Anim. Sci. 4:118–128. doi:10.1093/tas/txz164.

Polizel, D. M., M. F. Westphalen, A. A. Miszura, M. H., Santos, R. G. Silva, A. V. Bertoloni, G. B. Oliveira, M. V. Biehl, M. V. C. Ferraz Junior, I. Susin,et al. 2016. Effect of narasin on nutrient intake and digestibility wethers fed high-forage diets. J. Anim. Sci. 94 (E-Suppl. 5):807. doi: 10.2527/jam2016-1684

Ricke, S. C., L. L. Berger, P. J. Van der Aar, and G. C. Fahey. 1984. Effects of lasalocid and monensin on nutrient digestion, metabolism and rumen characteristics of sheep. J. Anim. Sci. 58:194–202. doi: 10.2527/jas1984.581194x

Rogers, M., J. P. Jouany, P. Thivend, and J. P. Fontenot. 1997. The effects of short-term and long-term monensin supplementation, and its subsequent withdrawal on digestion in sheep. Anim. Feed Sci. Technol. 65:113–127. doi:10.1016/S0377-8401(96)01089-9.

Russel, J. B., and H. J. Strobel. 1989. Effect of lonophores on ruminal fermentation. Appl. Environ. Microbiol. 55:1–6. doi:10.1128/aem.55.1.1-6.1989.

Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32:199–208. doi:10.1079/bjn19740073

Sardinha, L. A., R. S. Marques, A. A. Miszura, J. P. R. Barroso, G. B. Oliveira, A. S. Martins, A. C. Limede, M. V. C. Ferraz Junior, E. M. Ferreira, A. V. Pires, M. L. Eastridge, D. M. Polizel. 2020. Milk yield and composition from ewes fed diets containing narasin and their lambs' performance. Transl. Anim. Sci. 4:1-9. doi: 10.1093/tas/txaa030

Schären, M., C. Drong, K. Kiri, S. Riede, M. Gardener, U. Meyer, J. Hummel, T. Urich, G. Breves, and S. Dänicke. 2017. Differential effects of monensin and a blend of essential oils on rumen microbiota composition of transition dairy cows. J. Dairy Sci. 100:2765–2783. doi:10.3168/jds.2016-11994.

Silva, R. G., M. V. Ferraz Junior, V. N. Gouveia, D. M. Polizel, M. H. Santos, A. A. Miszura, T. S. Andrade, M. F. Westphalen, M. V. Biehl, and A. V. Pires. 2015. Effects of narasin in mineral mix to Nellore heifers fed with high forage. J. Anim. Sci. 93 (Suppl. S3):118.

Slyter, L. L., L. D. Satter, and D. A. Dinius. 1979. Effect of ruminal ammonia concentration on nitrogen utilization by steers. J. Anim. Sci. 48:906–912.

Tedeschi, L. O., D. G. Fox, and T. P. Tylutki. 2003. Potential environmental benefits of ionophores in ruminant diets. J. Environ. Qual. 32:1591–1602. doi:10.2134/jeq2003.1591.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Volke, F., R. Waschipky, A. Pampel, A. Donnerstag, G. Lantzsch, H. Pfeiffer, W. Richter, G. Klose, and P. Welzel. 1997. Characterisation of antibiotic moenomycin A interaction with phospholipid model membranes. Chem. Phys. Lipids 85:115–123. doi:10.1016/S0009-3084(96)02649-7

Watson, A. K., J. C. MacDonald, G. E. Erickson, P. J. Kononoff, and T. J. Klopfenstein. 2015. Forages and pastures symposium: optimizing the use of fibrous residues in beef and dairy diets. J. Anim. Sci. 93:2616–2625. doi:10.2527/jas.2014-8780

Wedegaertner, T. C., and D. E. Johnson. 1983. Monensin effect on digestibility, methanogenesis and heat increment of cracked corn-silage diet fed to steers. J. Anim. Sci. 57: 168–177. doi:10.2527/jas1983.571168x.

Weimer, P. J., D. M. Stevenson, D. R. Mertens, and E. E. Thomas. 2008. Effect of monensin feeding and withdrawal on populations of individual bacterial species in the rumen of lactating dairy cows fed high-starch rations. Appl. Microbiol. Biotechnol. 80:135–145. doi:10.1007/s00253-008-1528-9.

Weiss, W. P., H. R. Conrad, and N. R. St. Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed

Sci. Technol. 39:95–110. doi: 10.1016/0377-8401(92)90034-4

Yokoyama, M. T., and K. A. Johnson. 1988. Microbiology of the rumen and intestine. In: D. C. Church editor. The ruminant animal, New York (NY): Simon and Shuster; p. 125–144.

Zinn, R. A. 1986. Influence of forage level on response of feedlot steers to salinomycin supplementation. J. Anim. Sci. 63:2005–2012. doi:10.2527/jas1986.6362005x.

Table 2. Nutritional profile of the Tifton-85 (Cynodon dactylon spp.) haylage and ground citrus pulp and ground corn mixed used in Exp. 1.[1]

	Period of Study[2]					
Item	**1**	**2**	**3**	**4**	**5**	**CI:C**[3]
Nutrient profile, dry matter basis						
Dry Matter	47.7	57.8	64.3	58.1	60.4	87.3
Crude Protein, %	18.9	18.0	18.8	16.1	16.0	7.80
Neutral Detergent Fiber, %	63.6	63.0	66.2	69.6	67.2	14.5
Acid Detergent Fiber, %	30.1	33.3	29.7	34.8	30.8	3.80
Extract Ether, %	2.91	2.16	2.48	2.19	2.73	2.60
Ash, %	12.3	11.8	9.29	10.2	10.0	4.91
Total Digestible Nutrients[4], %	53.9	53.8	55.7	54.1	55.5	81.9
Net energy of maintence[5], Mcal/kg	1.10	1.10	1.17	1.11	1.16	2.00
Net energy of gain[5], Mcal/kg	0.55	0.54	0.61	0.55	0.60	1.35

[1] Based on nutritional profile of each ingredient, which were analyzed via wet chemistry procedures (AOAC, 1990).

[2] The experimental period lasted 140 days and was divided into 5 periods of 28 days each

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

[4] Calculations were performed according to the equations proposed by Weiss et al. (1992).

[5] Calculated composition using tabular values from NASEM (2016).

Table 3. Nutritional profile of the Tifton-85 (Cynodon dactylon spp.) haylage and ground corn (GC) used in Exp. 2.[1]

Item	Period of Study[2]					GC
	1	2	3	4	5	
Nutrient profile, dry matter basis						
Dry Matter	37.4	48.0	46.9	49.0	49.7	88.0
Crude Protein, %	21.0	23.2	19.3	17.0	13.3	9.18
Neutral Detergent Fiber, %	57.8	56.3	61.3	59.3	61.8	12.6
Acid Detergent Fiber, %	29.2	30.0	28.7	29.6	34.8	4.59
Extract Ether, %	2.54	2.26	3.60	2.40	2.01	3.91
Ash, %	14.1	10.3	13.1	11.1	10.4	1.50
Total Digestible Nutrients[3], %	52.8	54.4	54.4	55.8	55.9	88.9
Net energy of maintence[4], Mcal/kg	1.07	1.18	1.12	1.17	1.17	2.21
Net energy of gain[4], Mcal/kg	0.51	0.62	0.56	0.61	0.61	1.52

[1] Based on nutritional profile of each ingredient, which were analyzed via wet chemistry procedures (AOAC, 1990).

[2] The experimental period lasted 140 days and was divided into 5 periods of 28 days each

[3] Calculations were performed according to the equations proposed by Weiss et al. (1992).

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

Table 4. Intake and apparent digestibility of nutrients of Bos indicus Nellore steers receiving a high forage-based diets supplemented or not (**CON**, n = 8) with narasin (**NAR**, n = 8), salinomycin (**SAL**; n = 8), and flavomycin (**FLA**; n =8) for 140 days.

	Treatments[1]				SEM	*P* Value[2]		
Item	**CON**	**NAR**	**SAL**	**FLA**		**Treatment**	**Period**	**T × P**
Intake, kg/day								
Dry Matter	5.93	5.85	5.71	5.45	0.26	0.20	<0.01	0.33
Organic Matter	5.32	5.24	5.09	4.87	0.23	0.17	<0.01	0.39
Crude Protein	1.05	1.03	1.01	0.97	0.44	0.20	<0.01	0.43
Neutral Detergent Fiber	3.99	3.94	3.85	3.67	0.17	0.18	<0.01	0.35
Acid Detergent Fiber	1.88	1.86	1.81	1.73	0.08	0.19	<0.01	0.33
Digestibility, % (dry matter basis)[3]								
Dry Matter	52.39	53.14	52.07	53.26	1.08	0.80	<0.01	0.70
Organic Matter	57.19	57.86	56.73	58.10	0.96	0.70	<0.01	0.83
Crude Protein	63.70	64.31	63.39	63.96	0.93	0.90	<0.01	0.70
Neutral Detergent Fiber	60.27	61.07	58.78	61.06	1.06	0.40	<0.01	0.48
Acid Detergent Fiber	54.59	55.83	52.90	55.35	1.28	0.40	<0.01	0.58

[1] CON = no feed additives; NAR = inclusion of 13 ppm of narasin (Zimprova, Elanco Animal Health, São Paulo, Brazil); SAL= inclusion of 20 ppm of salinomycin (Posistac, Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil); FLA = inclusion of 3 ppm of flavomycin (Flavomycin 80, Huvepharma, Porto Alegre, Rio Grande do Sul, Brazil). Within rows, values with different superscripts differ ($P \leq 0.05$).

[2] *P* Value for Treatment, Period and Treatment × Period interaction (T × P).

[3] From d 23 to 27 (period 1), 51 to 55 (period 2), 79 to 83 (period 3), 107 to 111 (period 4), and 135 to 139 (period 5), total fecal production was individually collected to determine apparent nutrient digestibility analysis. Apparent digestibility was calculated according to the formula: TTAD

(%) = ((DMI × NCDM) – (FDM × NCFM) × 100) / (DMI × NCDM), where TTAD = total tract apparent digestibility, DMI = dry matter intake, NCDM = nutrient content of the DMI (%), FDM = fecal dry matter, and NCFM = nutrient content of the fecal DM (%).

Table 5. Rumen volatile fatty acids (VFA) concentrations, ammonia, and pH of Bos indicus Nellore steers receiving a high forage-based diets supplemented or not (**CON**, n = 8) with narasin (**NAR**, n = 8), salinomycin (**SAL**; n = 8), and flavomycin (**FLA**; n =8) for 140 days (Exp.1).

	Treatments[1]					***P*-Value**[2]		
Item	**CON**	**NAR**	**SAL**	**FLA**	**SEM**[2]	**Treatment**	**Day**	**T × D**
Volatile fatty acids, mM/100mM[3]								
Acetate	73.46^{b}	72.98^{a}	73.89^{c}	73.54^{bc}	0.14	<0.01	<0.01	0.34
Propionate	13.77^{b}	14.53^{a}	13.49^{b}	13.43^{b}	0.14	<0.01	<0.01	0.17
Isobutyrate	1.01	1.07	1.05	1.07	0.03	0.44	<0.01	0.59
Butyrate	9.05^{c}	8.60^{a}	8.73^{ab}	8.97^{bc}	0.10	0.01	<0.01	0.79
Isovalerate	1.52	1.58	1.54	1.66	0.04	0.12	<0.01	0.45
Valerate	1.26	1.23	1.28	1.27	0.02	0.23	<0.01	0.82
Ac:Pr	5.39^{b}	5.01^{a}	5.49^{b}	5.49^{b}	0.05	<0.01	<0.01	0.11
AcBut:Pr	5.98^{b}	5.65^{a}	6.04^{b}	6.06^{b}	0.05	<0.01	<0.01	<0.01
Total VFA, mM	53.32^{a}	51.96^{a}	41.11^{b}	42.32^{b}	3.02	0.02	<0.01	0.81
Ammonia, mg/dL	3.10	2.93	3.38	3.43	0.21	0.29	<0.01	0.53
Rumen pH	6.76	6.89	6.88	6.80	0.05	0.28	<0.01	0.54

[1]CON = no feed additives; NAR = inclusion of 13 ppm of narasin (Zimprova, Elanco Animal Health, São Paulo, Brazil); SAL= inclusion of 20 ppm of salinomycin (Posistac, Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil); FLA = inclusion of 3 ppm of flavomycin (Flavomycin 80, Huvepharma, Porto Alegre, Rio Grande do Sul, Brazil). Within rows, values with different superscripts differ ($P \leq 0.05$). Ac:Pr = acetate:propionate ratio; AcBut:Pr = acetatebutirate:propionate ratio

[2]*P* Value for Treatment, Day and Treatment × Day interaction (T × D).
[3] 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 supplement + treatments, ruminal fluid samples were collected (approximately 100 mL).

Table 6. Performance of Bos indicus Nellore bulls receiving control (without feed additive; **CON**, n = 8), narasin (**NAR**, n = 8), salinomycin (**SAL**; n = 8), and flavomycin (**FLA**; n = 8), in high forage-based diets for 140 days.

	Treatments[2]				**SEM**	***P* – value**[3]		
Item[1]	**CON**	**NAR**	**SAL**	**FLA**		**Treatment**	**Period**	**T × P**
Body weight, kg								
Initial (day 0)	298.9	299.2	298.9	298.9	2.50	0.99	-	-
Day 28	309.7	314.6	309.1	311.8	2.56	0.41	-	-
Day 56	347.7	354.8	347.2	351.3	2.56	0.13	-	-
Day 84	369.8^{b}	378.4^{a}	368.8^{b}	374.1^{ab}	2.56	0.03	-	-
Day 112	391.8^{b}	403.9^{a}	392.9^{b}	397.2^{ab}	2.56	<0.01	-	-
Final (d 140)	409.7^{b}	424.2^{a}	406.4^{b}	414.8^{b}	2.52	0.02	-	-
DMI, kg	6.42^{b}	6.93^{a}	6.26^{b}	6.37^{b}	0.14	0.04	<0.01	0.03
DMI, % BW	1.82^{b}	1.93^{a}	1.76^{b}	1.78^{b}	0.15	0.01	<0.01	0.08
ADG, kg	0.791^{b}	0.908^{a}	0.812^{b}	0.842^{b}	0.02	<0.01	<0.01	0.73
G:F, g/kg	119.6	126.6	125.8	127.9	4.14	0.51	<0.01	0.95

[1] DMI = dry matter intake; DMI, % BW = dry matter intake percentage of body weight; ADG = average daily gain; G:F = feed efficiency. On d 0 of the experimental period, individual shrunk BW was recorded after 14 h of feed and water withdrawal to determine animal initial body weight. To calculate ADG and 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. Dry matter intake was evaluated daily from each pen within each period by collecting and weighing non-consumed feed weekly. Hay and total DMI of each pen were divided by the number of bulls within each pen and expressed as kg per bull/day. Total BW gain and DMI of each period were used for bull G:F calculation.

[2] CON = no feed additives; NAR = inclusion of 13 ppm of narasin (Zimprova, Elanco Animal Health, São Paulo, Brazil); SAL= inclusion of 20 ppm of salinomycin (Posistac, Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil); FLA = inclusion of 3 ppm of flavomycin (Flavomycin 80, Huvepharma, Porto Alegre, Rio Grande do Sul, Brazil). Within rows, values with different superscripts differ ($P \leq 0.05$).

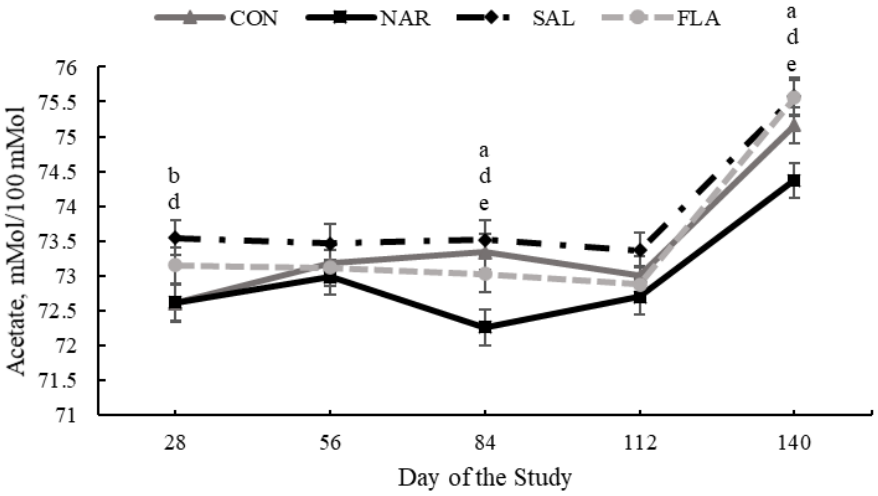
[3] *P* Value for Treatment, Period, and Treatment × Period interaction (T × P).

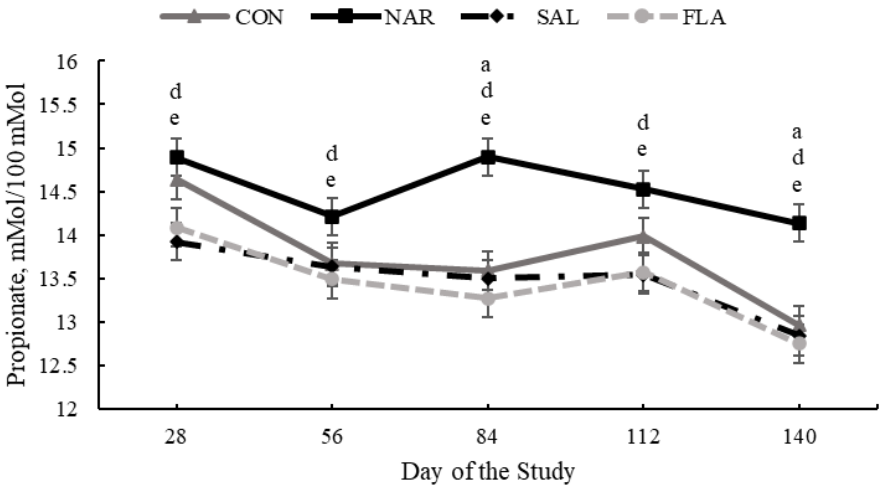
Figure 2. Molar concentration of acetate (Panel A), propionate (Panel B), Acetate:Propionate ratio (Panel C), and AcetateButyrate:Propionate ratio (Panel D) of *Bos indicus* Nellore steers receiving a high forage-based diets supplemented or not (**CON**, $n = 8$) with 13 ppm of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil; **NAR**, $n = 8$), 20 ppm of salinomycin (Posistac, Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil; **SAL**, $n = 8$), or 3 ppm of flavomycin (Flavomycin 80, Huvepharma, Porto Alegre, Rio Grande do Sul, Brazil; **FLA**, $n = 8$). Treatments were offered daily throughout the experimental period (day 0 to 140). Rumen samples were collected on day 0 (prior to first treatment administration), 28, 56, 84, 112, and 140 of the study. Data were analyzed using results from d 0 as independent covariate. For all figures below, within days, letters indicate treatment comparisons ($P \leq 0.05$): a = CON vs. NAR, b = CON vs. SAL, c = CON vs. FLA, d = NAR vs. SAL, e = NAR vs. FLA, and f = SAL vs. FLA.

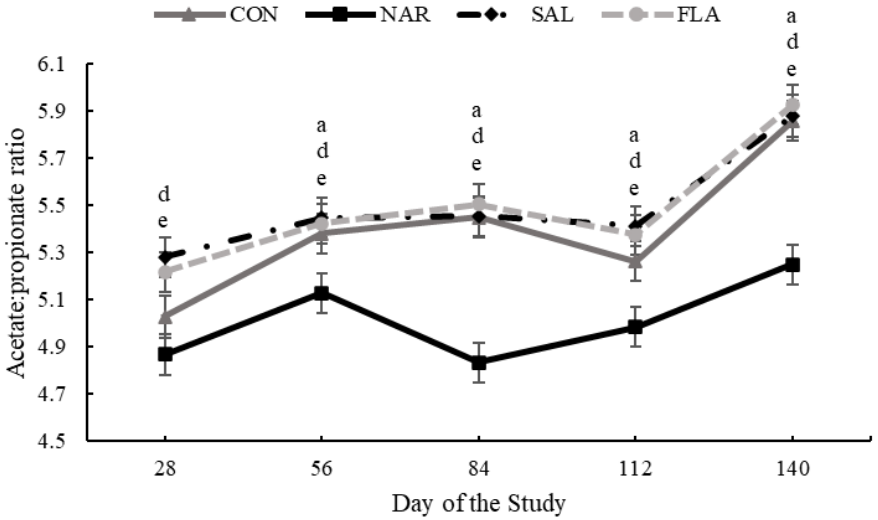
Figure 3. Body weight of *Bos indicus* Nellore bulls receiving a high forage-based diets supplemented or not (**CON**, $n = 8$) with 13 ppm of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil; **NAR**, $n = 8$), 20 ppm of salinomycin (Posistac, Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil; **SAL**, $n = 8$), or 3 ppm of flavomycin (Flavomycin 80, Huvepharma, Porto Alegre, Rio Grande do Sul, Brazil; **FLA**, $n = 8$). Treatments were offered daily throughout the experimental period (day 0 to 140). Body weight was recorded on day 0 (prior to first treatment administration), 28, 56, 84, 112, and 140 of the study after 14 h of feed and water withdrawal. Within days, letters indicate treatment comparisons ($P \leq 0.05$): a = CON vs. NAR, b = CON vs. SAL, c = CON vs. FLA, d = NAR vs. SAL, e = NAR vs. FLA, and f = SAL vs. FLA.

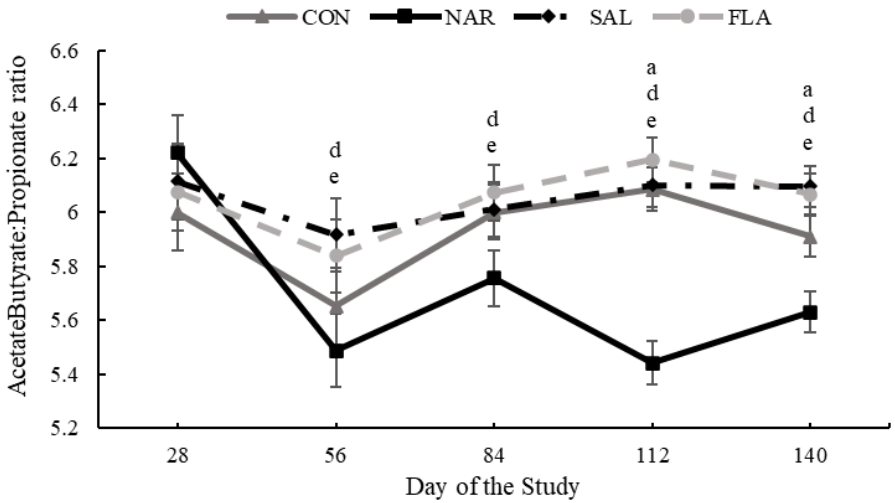
Figure 4. Dry matter intake of *Bos indicus* Nellore bulls receiving a high forage-based diets supplemented or not (**CON**, $n = 8$) with 13 ppm of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil; **NAR**, $n = 8$), 20 ppm of salinomycin (Posistac, Phibro

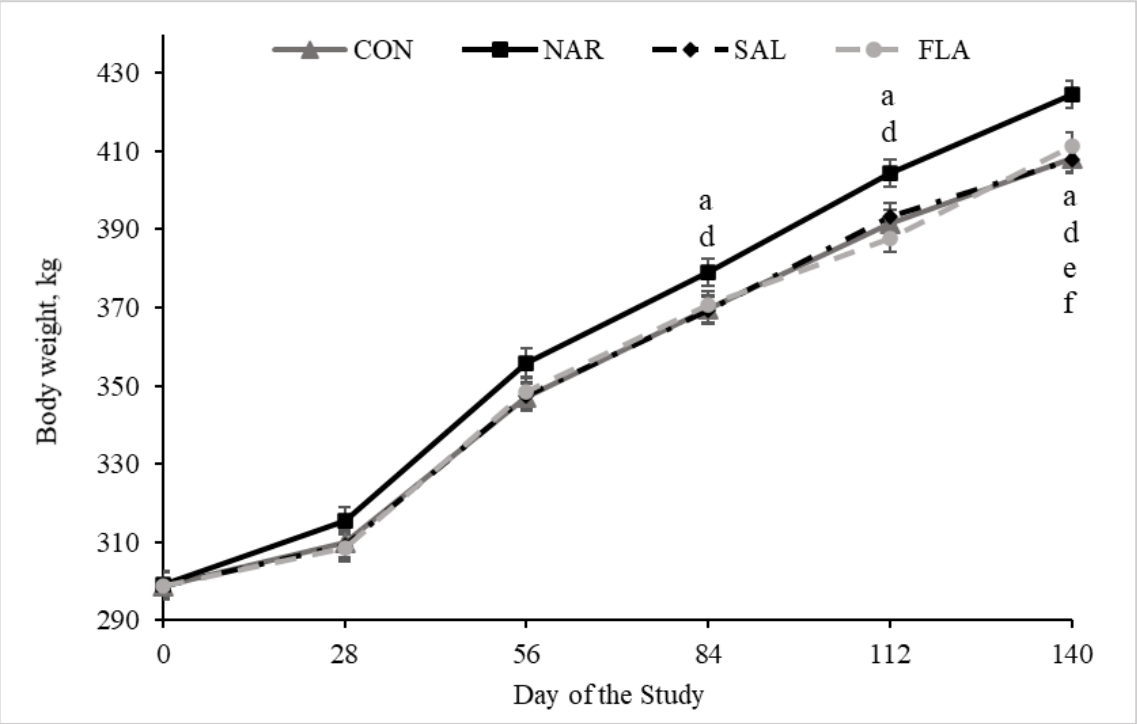
Animal Health Corporation, Guarulhos, São Paulo, Brazil; **SAL**, *n* = 8), or 3 ppm of flavomycin (Flavomycin 80, Huvepharma, Porto Alegre, Rio Grande do Sul, Brazil; **FLA**, *n* = 8). Treatments were offered daily throughout the experimental period (day 0 to 140). Dry matter intake was evaluated daily from each pen within each period by collecting and weighing non-consumed feed weekly. Hay and total dry matter intake of each pen were divided by the number of bulls within each pen and expressed as kg/bull per day. Within days, letters indicate treatment comparisons ($P \leq 0.05$): a = CON vs. NAR, b = CON vs. SAL, c = CON vs. FLA, d = NAR vs. SAL, e = NAR vs. FLA, and f = SAL vs. FLA.

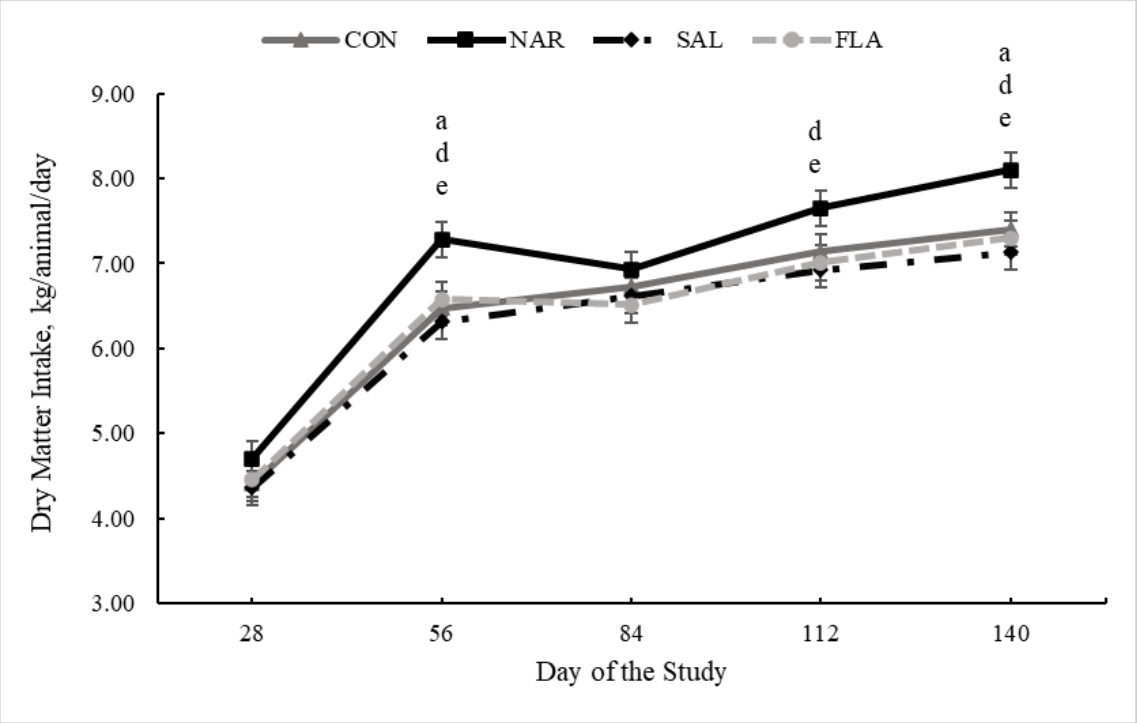

CON
NAR
SAL
FLA
Acetate, mMol/100 mMol
Day of the Study


CON
NAR
SAL
FLA
Propionate, mMol/100 mMol
Day of the Study


CON
NAR
SAL
FLA
Acetate:propionate ratio
Day of the Study


CON
NAR
SAL
FLA
AcetateButyrate:Propionate ratio
Day of the Study


CON
NAR
SAL
FLA
Body weight, kg
430
410
390
370
350
330
310
290
0
28
56
84
112
140
Day of the Study
a
d
a
d
a
d
e
f


CON
NAR
SAL
FLA
Dry Matter Intake, kg/animal/day
9.00
8.00
7.00
6.00
5.00
4.00
3.00
a
d
e
d
e
a
d
e
28
56
84
112
140
Day of the Study