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Effect of forage quality and narasin inclusion on ruminal fermentation, nutrient intake, and total tract digestibility of Nellore steers

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ABSTRACT

The study aimed to evaluate the effects of forage quality and narasin inclusion on intake, digestibility, and ruminal fermentation of Nellore steers. Twenty-eight rumen-cannulated Nellore steers (initial body weight [**BW**] = 350 ± 32.4 kg) were allocated to individual pens in a randomized complete block design, with 7 blocks, defined according to the fasting BW at the beginning of the experiment. The steers were randomly assigned within blocks to 1 of 4 experimental diets in 2 × 2 factorial arrangements, being the first-factor forage quality (**MEDIUM** = 81 g of CP/kg of dry matter [**DM**], and **HIGH** = 153 g of CP/kg of DM), and the second factor was the inclusion (**N13** = diet plus 13 mg/kg of DM of narasin) or not (**N0**) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil). The experiment consisted of a 28-d period with 22 d for adaptation and the last 6 d for data collection. No haylage quality × narasin interaction ($P \geq 0.68$) was observed on DM and nutrient intake. Haylage quality affected ($P \leq 0.01$) DM intake, with greater values observed for steers fed HIGH compared with MEDIUM haylage. There was an increase ($P < 0.001$) in OM, NDF, hemicellulose, and CP intake for steers consuming HIGH vs. MEDIUM haylage. Including N13 did not affect ($P > 0.39$) DM and nutrient intake of steers. No haylage quality × narasin interactions were detected ($P \geq 0.60$) for total tract nutrient digestibility. However, steers fed with HIGH haylage showed an increase ($P > 0.001$) in DM and digestibility of all nutrients compared with MEDIUM. Steers fed a MEDIUM haylage had a greater ($P < 0.01$) proportion of acetate compared with steers fed HIGH during all evaluated hours. Steers fed HIGH haylage had a greater ($P < 0.01$) proportion of propionate at 0 h compared with steers consuming MEDIUM, whereas at 12 h, steers consuming MEDIUM hay had a greater ($P < 0.01$) proportion of propionate vs. HIGH haylage. A haylage quality × narasin and haylage quality × time of collection interactions were detected ($P \leq 0.03$) for rumen ammonia concentration, which was reduced ($P < 0.03$) in N13 vs. N0 steers consuming HIGH haylage. Collectively, high-quality haylage allows increased consumption and digestibility, with more energy-efficient ruminal fermentation. In addition, narasin might be an important nutritional tool in forage-based diets to enhance the ruminal fermentation parameters of *Bos indicus* Nellore steers.

LAY SUMMARY

In temperate and tropical climates, beef cattle production frequently depends on diets based on forage. Seasonal fluctuations in pasture quality and quantity severely impede the usage of nutrients by grazing animals. To meet the animal's nutrient requirements, nutritional managements are routinely utilized to reduce the imbalanced dietary composition of the forage, optimizing intake and nutrient digestibility. Narasin is an ionophore that improves growth and alters ruminal fermentation of beet cattle consuming forage-based diets. This experiment evaluated the interaction between forage quality and dietary inclusion of narasin of *Bos indicus* Nellore steers. Steers consuming high-quality haylage had greater nutrient intake and digestibility than steers consuming medium-quality haylage. Haylage quality also altered ruminal short-chain fatty acids (**SCFA**), favoring steers consuming high-quality haylage. An interaction between haylage quality and narasin inclusion was noted for total SCFA, valerate, and rumen ammonia, which was reduced in steers consuming narasin and high-quality haylage. Narasin inclusion increased propionate, isobutyrate, acetate:propionate, and acetate-butyrate:propionate ratio regardless of haylage quality. Overall, narasin effectively improved ruminal fermentation efficiency, implying that it could be used as a nutritional alternative in grazing systems even when the forage composition varies. Furthermore, forage quality had a significant influence on intake, digestibility, and ruminal fermentation.

Key words: feed additives, forage quality, ionophore, narasin, ruminal fermentation

INTRODUCTION

Beef cattle production in tropical and temperate regions often depends on diets based on forage. Nevertheless, seasonal variations in pasture quality and quantity significantly impair the use of nutrients that benefit the performance and intake of grazing animals (Hills et al., 2015; de Souza et al., 2017). Additionally, forage composition has variable impacts on feed intake and rumen fermentation parameters, which

could negatively impact the performance of grazing animals (Sutton, 1989; Beauchemin and Yang, 2005). Hence, nutritional strategies are frequently used to reduce the imbalanced nutritional composition of the forage and optimize intake and ruminal fermentation parameters in order to meet the animal nutrient requirements (NASEM, 2016; Limede et al., 2021).

Voluntary intake of ruminants depends on several factors, including the digestion rate of the nutrients, the volume occupied by the fermentable ingredients of the diet, and the rate at which those nutrients are digested in the rumen (Hungate, 1966; Allison, 1985). One of the most important variables in forage-based diets is fiber digestibility, which can be influenced by a variety of factors such as fiber quality, NDF intake quantity, and interaction with other nutrients (i.e., protein) or nutritional tools (i.e., feed additives) in the diet (Van Soest, 1994; Souza et al., 2010).

Using ionophores in ruminant feed is an effective nutritional strategy to manipulate ruminal fermentation and consequently increase nutrient digestibility (Marques and Cooke 2021). Ionophores can modulate rumen fermentation patterns by altering the molar proportions of short-chain fatty acids (SCFA) produced in the rumen. Using ionophores also results in better energy retention efficiency, use of nitrogen from the diet, mitigation of methane production, and lower occurrence of metabolic disorders (McGuffey et al., 2001). Narasin is a monovalent ionophore that increases the concentration of ruminal propionate and total SCFA and reduces acetate:propionate (Ac:Prop) ratio of animals consuming forage-based diets (Polizel et al., 2020; Limede et al., 2021; Miszura et al., 2023). However, none of our earlier studies demonstrated an improvement in digestibility when narasin was given to animals eating diets based on forage, despite the fact that the forage quality varied between these studies (Polizel et al., 2020—average CP = 10.3%; Limede et al., 2021—average CP = 17.6%; Soares et al., 2021—CP = 20.8%; Miszura et al., 2023—average = CP 12%). Additionally, there are no reports in the literature evaluating the interaction of forage quality and narasin inclusion on intake, rumen fermentation, and nutrient digestibility. Hence, our research group hypothesizes that narasin may have varying effects on dry matter intake (DMI), nutrient digestibility, and ruminal fermentation parameters depending on forage quality. Our objective was to evaluate the effects of forage quality and narasin inclusion on the intake, digestibility, and ruminal fermentation of *Bos indicus* Nellore steers.

MATERIAL AND METHODS

This study was carried out at the Laboratory of Animal Nutrition and Reproduction, Animal Science Department, "Luiz de Queiroz" College of Agriculture, University of Sao Paulo, located in Piracicaba, Sao Paulo, Brazil. The animal care and use committee from the University of Sao Paulo approved all animal procedures (protocol number 1946311019).

Animal, Experimental Design, and Diets

Twenty-eight cannulated *Bos indicus* Nellore steers (initial body weight [BW] = 350 ± 32.4 kg; age = 20 ± 1 mo) were assigned to individual pens (2.5 × 4.5 m; concrete surface, with a waterer, feed bunk, and mineral supplement bunk; pens protected from rain and direct sunlight) in a randomized complete block design according to the initial shrunk BW. Within each block (*n* = 4), steers were randomly assigned to receive 1

of 4 treatments in a 2 × 2 factorial arrangement of treatments. As a result, 4 treatment combinations were generated, being the first-factor forage quality (MEDIUM with 81 g of CP/kg of dry matter [DM]; *n* = 7; HIGH with 153 g of CP/kg of DM; *n* = 7), and the second factor was the inclusion (N13 = diet plus 13 mg/kg of DM of narasin; *n* = 7) or not (N0; *n* = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil. The dosage used herein was according to the manufacturer's recommendation and also was tested by our research group (Silva et al., 2015; Polizel et al., 2020, 2021). The proportion of ingredients and chemical composition are presented in Table 1.

Throughout the experiment, steers were fed 99% of Tifton-85 haylage (*Cynodon dactylon* spp.) and 1% of ground corn, which was used as a delivery vehicle for narasin (N13). However, the steers that received the N0 treatment were fed the same proportion of ground corn without including narasin. Haylage was chopped daily using a vertical mixer (Mixer VM8B, DeLaval International AB, Rumba, Sweden), and the corn was ground using a grinder (Nogueira DPM—4, Itapira, Sao Paulo, Brazil) equipped with a 10-mm pore size sieve. Narasin was weighed on an analytical balance with a precision of 0.0001 g (Sartorius BA11OS, Gottingen, Germany) and premixed with part of the ground corn. Subsequently, the premixed was homogenized with the total amount of corn using a horizontal mixer (Lucato, Limeira, Sao Paulo, Brazil). The concentrate was weighed and offered to each animal daily (0800 hours), before forage supply (0830 hours) to avoid the small amount of supplement being mixed with haylage and compromising the immediate intake of the concentrate, and consequently, the narasin (N13). Steers promptly consumed the concentrate within 10 min after supply. Animals had free access to fresh water and mineral mix, offered in a separate bunk, so there was no mixing with the concentrate and haylage. 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 experiment consisted of a 28-d period with 22 d for adaptation and the last 6 d for data collection. The nutritional profile of the haylage and ground corn used herein is described in Table 1.

Data Collection

Individual shrunk BW was obtained on days 0 and 29 after 14 h of feed withdrawal to determine initial and final BW, in which the initial BW was used to perform the randomization into blocks and treatments. The final BW was used to calculate the average BW to evaluate the DM intake in kg/d, % BW, and g/kg of $BW^{0.75}$. From days 23 to 27, total fecal production was collected from the ground and quantified twice daily (0800 and 1800 hours) using an electronic scale (Marte AC-10K; Marte Cientifica, Sao Paulo, SP, Brazil). A representative sample of the total fecal (10%) was taken from each steer and stored at –18 °C for further chemical analysis. The experimental pens had a specific drainage system with a 1% slope to avoid feces contamination with urine. In this same period, the feed and orts of each steer were weighed daily and sampled for subsequent chemical analysis and calculation of nutrient intake. Total tract apparent nutrient digestibility was calculated as described by Soares et al. (2021): TTAD (%) = [(DMI × NCDM) – (FDM × NCFM) × 100]/[DMI × NCDM], where TTAD = total tract apparent

Table 1. Nutrient profile of the Tifton-85 haylage and ground corn

Item[1]	Haylage		Ground corn
	HIGH	**MEDIUM**	
DM, g/kg			
DM, as fed	257	393	885
Organic matter	900	908	987
Neutral detergent fiber	637	754	108
Acid detergent fiber	371	577	30.5
Hemicelluloses	266	177	77.5
Celluloses	299	474	20.0
Lignin	72.0	103	10.5
CP	153	81	101
Neutral detergent insoluble nitrogen	41.8	30.1	8.0
Acid detergent insoluble nitrogen	22.6	17.9	2.2
Calculated energy[2]			
Total digestible nutrients, %	56.7	49.5	89.6
Dietary metabolizable energy, Mcal/kg	2.05	1.79	3.24
Net energy for maintenance, Mcal/kg	1.20	0.95	2.23
Net energy for gain, Mcal/kg	0.64	0.40	1.54

[1]Based on the nutritional profile of each ingredient, which was analyzed via wet chemistry procedures (AOAC, 1990).
[2]Calculated composition according to NASEM (2016) equations.

digestibility, NCDM = nutrient content of the DMI (%), FDM = fecal dry matter, and NCFM = nutrient content of the FDM (%).

On day 28 of the study, rumen fluid samples were collected at 0, 6, and 12 h after feeding. At each collection time, a sample of approximately 350 g of ruminal contents was collected via rumen cannula, quickly filtered on a 150-µm nylon cloth, and the liquid phase obtained was used for pH determination using a digital pH meter (Digimed DM20, Sao Paulo, Sao Paulo, Brazil). After filtering, the solid fraction remaining in the nylon cloth was returned to the rumen. Four aliquots of ruminal fluid were taken and stored at –18 °C for future determination of the SCFA and ruminal ammonia.

Sample Analysis

Haylage samples, ground corn, orts, and feces were thawed and dried in a forced-air oven at 55 °C for 96 h (AOAC, 1990; method #930.15). After initial drying, the samples were ground using a Willey Mill fitted with sieves with 1-mm sieves (Marconi, Piracicaba, Sao Paulo, Brazil). The DM content was determined by oven-drying the samples at 105 °C for 24 h (AOAC, 1990; method #934.01), and ash concentration was determined by incinerating the samples in an oven at 550 °C for 4 h (AOAC, 1990; method#942.05). The organic matter was calculated by the difference between 100 and the ash content. The determination of the fiber content (NDF and ADF) was performed as proposed by Van Soest et al. (1991) and Goering and Van Soest (1970), using an Ankom 2000 fiber analyzer. The sodium sulfite and heat-stable alpha-amylase were added to the NDF analysis. The difference between the NDF and ADF was used to calculate hemicellulose concentration. Total N was determined according to AOAC (1990; method #968.06) using the Leco TruMac N (Leco Corporation, St. Joseph, MI, USA), and the crude protein (**CP**) was calculated multiplying the total N content by 6.25.

Ruminal fluid samples were thawed and centrifuged ($15,000 \times g$) for 60 min at 4 °C, and SCFA determination was performed as described by Ferreira et al. (2016), quantifying the molar ratio (mM/100mM) of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate. In addition, acetate:propionate (**Ac:Prop**), acetate-butyrate:propionate ratio (**AcBut:Prop**), and total SCFA were calculated. The analysis of rumen ammonia concentration was performed according to procedures described by Broderick and Kang (1980) using a microplate reader (BIO-RAD, Hercules, CA) with a 550-nm absorbance filter.

Statistical Analysis

All data were analyzed for normality of residuals using the Shapiro–Wilk test, homogeneity of variances using the Levene test, and removal of outliers based on the student's r value. All data were analyzed using Kenward–Roger approximation to determine the denominator df for the test of fixed effects. Data were analyzed as a 2×2 factorial arrangement of treatments (haylage quality × narasin inclusion). The animal was considered the experimental unit, and the statistical model included the fixed effect of haylage quality (HIGH or MEDIUM), narasin inclusion (N0 or N13), and their interaction. The block was considered as a random effect. The specified term for all repeated statements was hour (ruminal fermentation parameters), with animal (treatment) as a subject. The covariance structure adopted was first-order autoregressive, which provided the smallest Akaike Information Criterion for the variables analyzed. The power analysis was performed using PROC POWER procedure of SAS (SAS Inst. Inc.; Cary, NC). The analysis was based on an independent samples t-test, aimed at detecting differences between treatment groups. The following parameters were used: a total sample size of 7 per group and an alpha level of 0.05, which achieved a power of ≥0.76 for all variables. All results 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. Results are reported according to the main effects if no interactions were significant.

RESULTS

No haylage quality × narasin inclusion interaction ($P \geq 0.68$) was observed on DM (%BW or g/kg of $BW^{0.75}$) and nutrient intake (Table 2). Haylage quality affected ($P \leq 0.01$) DM intake when evaluated in kg/d, % BW, and g/kg of $BW^{0.75}$, with greater values observed for steers fed HIGH than MEDIUM haylage. Consequently, there was an increase ($P < 0.001$) in OM, NDF, hemicellulose, and CP intake for steers consuming HIGH compared with MEDIUM haylage. No haylage quality effect was detected ($P = 0.64$) on ADF intake. The inclusion of N13 did not affect ($P > 0.39$) DM and nutrient intake of steers in this experiment. No haylage quality × narasin inclusion interactions were detected ($P \geq 0.60$) for total tract nutrient digestibility (Table 2). However, steers fed with HIGH haylage showed an increase ($P > 0.001$) in DM, and all nutrients digestibility was evaluated herein. There was no effect ($P \geq 0.39$) of narasin inclusion on total tract nutrient digestibility.

No haylage quality × narasin × time of collection interactions were detected ($P \geq 0.13$) for rumen fermentation variables (Table 3). Furthermore, there was no interaction ($P \geq 0.26$) between narasin inclusion and time (Table 3). However, haylage quality × time of collection interactions was observed ($P < 0.01$) for acetate, propionate, butyrate, isovalerate, valerate, Ac:Prop ratio, and AcBut:Prop ratio

(Table 3). Steers fed a MEDIUM haylage had a greater ($P < 0.01$) proportion of acetate compared with steers fed HIGH during all evaluated hours (Fig. 1). On the other hand, steers fed HIGH haylage had greater ($P < 0.01$) proportion of propionate at 0 h compared with steers consuming MEDIUM quality haylage, whereas at 12 h steers consuming MEDIUM haylage had greater ($P < 0.01$) proportion of propionate compared with steers fed HIGH, with no difference ($P \geq 0.26$) between haylage quality at 6 h (Fig. 2). Steers fed a HIGH haylage had a greater ($P < 0.01$) proportion of butyrate compared with steers fed MEDIUM haylage during all evaluated hours (Fig. 3). Steers fed a HIGH haylage also had greater ($P < 0.01$) proportions of isovalerate (Fig. 4) at 6 and 12 h, and valerate (Fig. 5) at 0 and 12 h compared with steers fed MEDIUM. Feeding steers with HIGH haylage only reduced ($P < 0.01$) the Ac:Prop ratio (Fig. 6) and AcBut:Prop ratio (Fig. 7) at 0 h.

A tendency for haylage quality × narasin inclusion interactions was noted ($P \leq 0.08$) for total SCFA and valerate, which was reduced ($P < 0.01$) in N13 vs. N0 steers consuming high-quality haylage, whereas the inclusion of narasin did not impact ($P > 0.36$) total SCFA of steers consuming medium-quality hay. Narasin inclusion increased ($P \leq 0.05$) propionate, isobutyrate, Ac:Prop, and AcBut:Prop ratio regardless of haylage quality. A haylage quality × narasin and haylage quality × time of collection interactions were detected ($P \leq 0.03$) for rumen ammonia concentration (Table 3). Ruminal ammonia concentration was reduced ($P < 0.03$) in N13 vs. N0 steers consuming high-quality haylage, whereas the inclusion of narasin did not impact ($P > 0.45$) ruminal ammonia concentration of steers consuming medium-quality hay. Steers consuming HIGH haylage had greater ($P < 0.01$)

Table 2. Nutrient intake and digestibility of nutrients in rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/kg of DM; *n* = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/kg of DM, *n* = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/kg of DM of narasin; *n* = 7) or not (N0; *n* = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil)

Item	Treatments				SEM	P Value[1]		
	HIGH		MEDIUM			Hay	Narasin	H × N
	N0	N13	N0	N13				
Intake								
DM, kg/d	5.48	5.52	4.22	4.13	0.21	<0.001	0.895	0.733
DM, % BW	1.53	1.51	1.19	1.17	0.06	<0.001	0.761	0.953
DM, g/kg $BW^{0.75}$	66.4	66.1	51.6	50.6	2.33	<0.001	0.791	0.889
Organic matter, kg/d	4.93	4.97	3.85	3.74	0.19	<0.001	0.852	0.701
Neutral detergent fiber, kg/d	2.92	2.94	2.62	2.56	0.12	0.007	0.857	0.720
Acid detergent acid, kg/d	1.52	1.53	1.57	1.53	0.06	0.648	0.846	0.683
Hemicellulose, kg/d	1.40	1.41	1.04	1.02	0.05	<0.001	0.870	0.727
CP, kg/d	0.75	0.75	0.31	0.30	0.03	<0.001	0.983	0.809
Digestibility[2], g/kg								
DM	562.4	542.2	426.9	404.0	26.57	<0.001	0.426	0.960
Organic matter	600.8	579.8	485.9	463.5	25.06	<0.001	0.395	0.977
Neutral detergent fiber	546.0	504.0	418.6	406.7	32.76	0.002	0.418	0.650
Acid detergent fiber	496.3	444.5	357.9	348.1	39.60	0.007	0.444	0.601
Hemicellulose	598.1	567.4	508.0	496.3	26.80	0.006	0.438	0.727
CP	607.0	601.7	328.0	335.2	21.57	<0.001	0.962	0.747

[1]*P* value for Hay, Narasin and Hay × Narasin interaction (H × N).
[2]Total tract apparent digestibility (%) = ([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 3. Molar proportion of short-chain fatty acids (SCFA), ammonia, and ruminal pH of rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/kg of DM; n = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/kg of DM, n = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/kg of DM of narasin; n = 7) or not (N0; n = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil)

Item	Treatments				SEM	P value[1]						
	HIGH		MEDIUM			Hay	Narasin	H × N	Time	H × T	N × T	H × N × T
	N0	N13	N0	N13								
Short-chain fatty acids, mM/100mM												
Acetate	75.7	75.3	77.8	77.1	0.30	<0.001	0.076	0.671	<0.001	0.017	0.263	0.206
Propionate	13.7	14.1	13.6	14.2	0.24	0.709	0.050	0.967	0.001	<0.001	0.725	0.368
Isobutyrate	1.25	1.36	0.96	1.02	0.04	<0.001	0.044	0.619	<0.001	0.135	0.788	0.560
Butyrate	6.86	6.49	5.59	5.45	0.17	<0.001	0.145	0.496	<0.001	<0.001	0.631	0.096
Isovalerate	1.74	1.66	1.38	1.43	0.06	<0.001	0.813	0.213	<0.001	<0.001	0.883	0.082
Valerate	0.94	0.84	0.65	0.69	0.04	<0.001	0.423	0.081	<0.001	0.007	0.874	0.180
Total, mM	80.9	74.2	67.3	68.9	2.14	0.001	0.237	0.064	<0.001	0.087	0.707	0.734
Ac:prop ratio[2]	5.55	5.35	5.67	5.43	0.10	0.361	0.049	0.889	<0.001	<0.001	0.972	0.377
AcBut:prop ratio[3]	6.06	5.82	6.07	5.82	0.11	0.977	0.036	0.934	<0.001	<0.001	0.867	0.344
pH	7.09	7.20	7.30	7.23	0.04	0.012	0.574	0.570	<0.001	0.394	0.297	0.831
Ammonia, mg/dL	11.5[a]	8.41[b]	6.12[b]	7.10[b]	0.93	0.001	0.266	0.037	<0.001	0.001	0.532	0.131

[1]P value for Hay, Narasin, Hay × Narasin interaction (H × N), Time, Hay × Time interaction (H × T), Narasin × Time interaction (N × T), and Hay × Narasin × Time interaction (H × N × T).
[2]Acetate:propionate molar proportion.
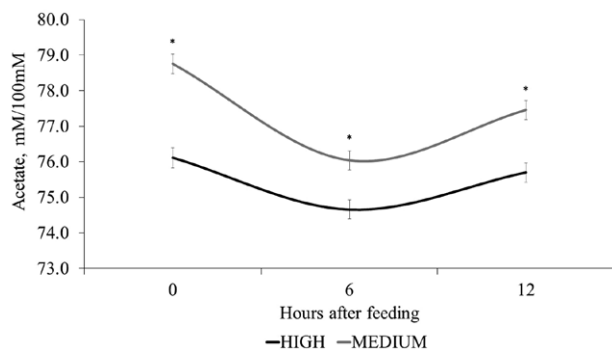[3]Acetate-butyrate:propionate molar proportion.

Figure 1. Molar proportion of acetate of rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/kg of DM; n = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/kg of DM, n = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/kg of DM of narasin; n = 7) or not (N0; n = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil). Treatments were offered daily throughout the experimental period (days 0 to 28). Rumen samples were collected on day 28 at 0, 6, and 12 h after feeding. Within the time of collection, the symbol (*) indicates significance (*P* ≤ 0.05) by hay quality.
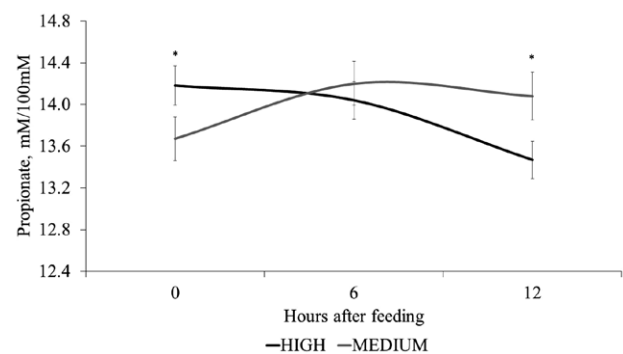

Figure 2. Molar proportion of propionate of rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/kg of DM; n = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/kg of DM, n = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/kg of DM of narasin; n = 7) or not (N0; n = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil). Treatments were offered daily throughout the experimental period (days 0 to 28). Rumen samples were collected on day 28 at 0, 6, and 12 h after feeding. Within the time of collection, the symbol (*) indicates significance (*P* ≤ 0.05) by hay quality.

ruminal ammonia concentration at 12 h compared with steers consuming MEDIUM haylage (Fig. 8). No treatment interactions were observed (*P* ≥ 0.29) for ruminal pH. However, ruminal pH was reduced (*P* < 0.01) in steers consuming HIGH compared with MEDIUM haylage. The inclusion of narasin did not impact (*P* = 0.26) ruminal pH of the steers.

DISCUSSION

During the annual production cycle, beef cattle production systems in tropical and temperate regions frequently rely on diets based on forage. However, seasonal fluctuations in pasture quality and quantity frequently impact beef cattle's ability to utilize nutrients and perform well by limiting their energy and protein intake (Hills et al., 2015; de Souza et al., 2017). Additionally, the voluntary intake of animals in forage-based systems might be impacted by several factors, including forage quality, passage rate, forage structure and composition, and rumen filling (Ellis et al., 1984; Van Soest, 1994). Accordingly, Mertens (1994) stated that the physical limitation, which is linked to the maximum distension or maximum volume or weight capacity of the ruminal cavity, is the factor that most interferes with the voluntary consumption of animals fed forage-based diets. Moreover, the ruminants voluntary intake is dependent on the digestion rate of the nutrient fractions, the volume occupied by the fermentable constituents of the diet, and how rapidly those
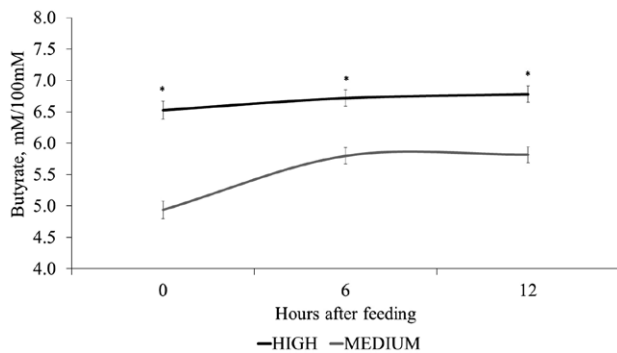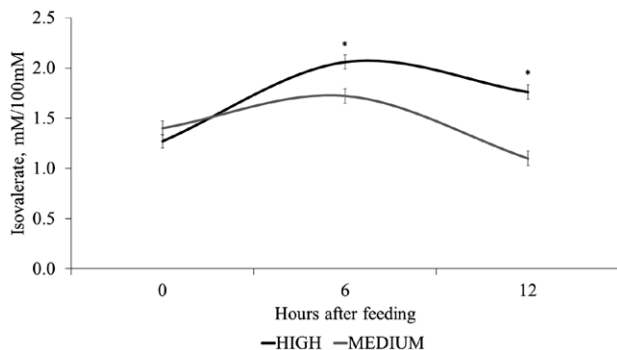
Figure 3. Molar proportion of butyrate of rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/kg of DM; *n* = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/kg of DM, *n* = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/kg of DM of narasin; *n* = 7) or not (N0; *n* = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil). Treatments were offered daily throughout the experimental period (days 0 to 28). Rumen samples were collected on day 28 at 0, 6, and 12 h after feeding. Within the time of collection, the symbol (*) indicates significance (*P* ≤ 0.05) by hay quality.

Figure 5. Molar proportion of valerate of rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/ kg of DM; *n* = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/ kg of DM, *n* = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/ kg of DM of narasin; *n* = 7) or not (N0; *n* = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil). Treatments were offered daily throughout the experimental period (days 0 to 28). Rumen samples were collected on day 28 at 0, 6, and 12 h after feeding. Within the time of collection, the symbol (*) indicates significance (*P* ≤ 0.05) by hay quality.

Figure 4. Molar proportion of isovalerate of rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/ kg of DM; *n* = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/ kg of DM, *n* = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/ kg of DM of narasin; *n* = 7) or not (N0; *n* = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil). Treatments were offered daily throughout the experimental period (days 0 to 28). Rumen samples were collected on day 28 at 0, 6, and 12 h after feeding. Within the time of collection, the symbol (*) indicates significance (*P* ≤ 0.05) by hay quality.

nutrients will be digested in the rumen-reticulum compartment (Hungate, 1966; Allison, 1985). One of the most significant variables in forage-based diets is the digestibility of the fiber fraction, which may be modified by several parameters such as fiber quality, the quantity of NDF intake, and interaction with other nutrients (i.e., protein) or nutritional tools (i.e., feed additives) available in the diet (Van Soest, 1994; Souza et al., 2010). In the current study, the use of high-quality haylage increased forage intake by approximately 30% compared with medium-quality haylage, which might be partially explained by the total amount of NDF and fiber composition (hemicellulose, cellulose, and lignin) of the haylages used herein. Accordingly, lignin is considered the main component that reduces the quality of the forage, diminishing nutrient intake and fiber digestibility (Moore and Mott, 1972). The haylages used herein had 72 and 103 g of lignin per kilogram of DM for high- and medium-quality haylage, respectively, which might explain the effects on the fibrous fraction digestibility (NDF and ADF), resulting in
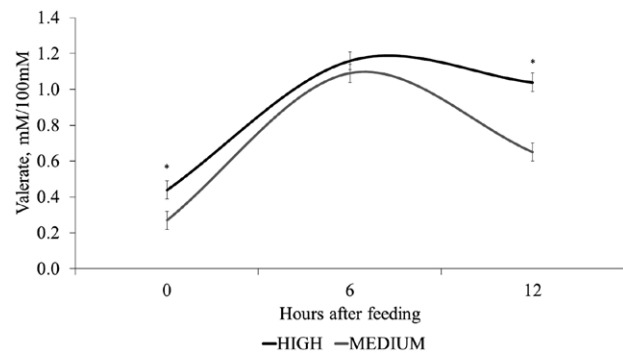
27% and 33% increase in NDF and ADF digestibility, respectively, when used a high-quality haylage. Furthermore, as NDF is the main constituent of the diet used herein, the reduction in the digestibility of this fraction would explain the decrease in DM and OM digestibility. Accordingly, Bohnert et al. (2011) mentioned that forage intake might increase in response to the interaction with other nutrients, especially protein supplementation offered to animals consuming low-quality forage. In the current study, animals offered high-quality haylage had an improvement in CP intake and digestibility by 146% and 82%, respectively, which might also explain the improvement in DM and OM intake and digestibility for animals consuming high-quality haylage. Two previous studies by our research group presented no difference in forage DMI when animals were fed with narasin in ruminal metabolism trials (Limede et al., 2021; Miszura et al., 2023). Nonetheless, the performance of both trials presented an increase in forage DMI intake when animals were fed with narasin. None of our previous experiments showed enhance in digestibility when animals consuming forage-based diets received narasin, even though between these experiments, the quality of the forage was different and very similar to the quality presented herein (Polizel et al., 2020—average CP = 10.3%; Limede et al., 2021—average CP = 17.6%; Soares et al., 2021—CP = 20.8%; Miszura et al., 2023—average CP = 12%). Comparing our results with others using different ionophores, no differences in apparent digestibility were observed when animals were offered a forage-based diet (Reffett-Stabel et al., 1989; DelCurto et al., 1998; Kobayashi et al., 1992). Kobayashi et al. (1992) found no variation in the apparent digestibility of nutrients in wethers treated with or without salinomycin. Consistent with those findings, Reffett-Stabel et al. (1989) noticed that salinomycin supplementation had no effect on total tract digestibility of nutrients when cattle received corn-silage-based diets. DelCurto et al. (1998) similarly observed that overall apparent NDF digestibility was not affected by monensin supplementation when cattle received forage-based diets. The lack of observed effects of narasin on intake and nutrient digestibility in animals consuming forage-based diets remains unexplored and warrants further investigation. It is possible
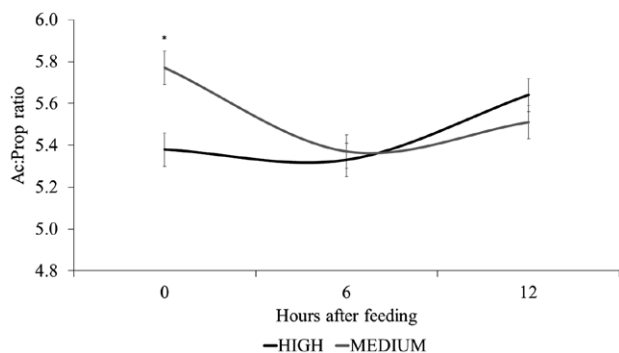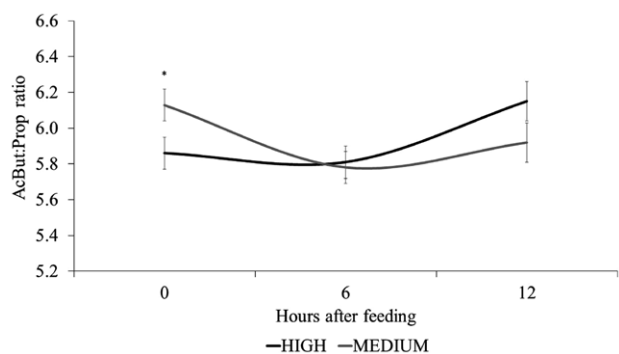
Figure 6. Molar proportion of acetate:propionate ratio of rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/ kg of DM; *n* = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/ kg of DM, *n* = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/kg of DM of narasin; *n* = 7) or not (N0; *n* = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil). Treatments were offered daily throughout the experimental period (days 0 to 28). Rumen samples were collected on day 28 at 0, 6, and 12 h after feeding. Within the time of collection, the symbol (*) indicates significance (*P* ≤ 0.05) by hay quality.

Figure 8. Ruminal ammonia concentration of rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/kg of DM; *n* = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/ kg of DM, *n* = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/ kg of DM of narasin; *n* = 7) or not (N0; *n* = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil). Treatments were offered daily throughout the experimental period (days 0 to 28). Rumen samples were collected on day 28 at 0, 6, and 12 h after feeding. Within the time of collection, the symbol (*) indicates significance (*P* ≤ 0.05) by hay quality.

Figure 7. Molar proportion of acetate-butyrate:propionate ratio of rumen-cannulated *Bos indicus* Nellore steers receiving HIGH (153 g of CP/kg of DM; 637 g of NDF/kg of DM; *n* = 7) or MEDIUM (81 g of CP/kg of DM; 754 kg of NDF/kg of DM, *n* = 7) quality Tifton-85 haylage with inclusion (N13 = 13 mg/kg of DM of narasin; *n* = 7) or not (N0; *n* = 7) of narasin (Zimprova; Elanco Animal Health, São Paulo, Brazil). Treatments were offered daily throughout the experimental period (days 0 to 28). Rumen samples were collected on day 28 at 0, 6, and 12 h after feeding. Within the time of collection, the symbol (*) indicates significance (*P* ≤ 0.05) by hay quality.

that the forage quality used in these studies influenced passage rate and gut fill, which may have impacted the response of ionophores (Ellis et al. 1984).

The lower CP digestibility presented by the medium quality (328 and 335 g/kg of DM) compared to high-quality haylage (607 and 602 g/kg of DM) may be associated with the composition of the protein. Medium-quality haylage in the present study had greater concentrations of NDIN and ADIN (Table 1), the latter being indigestible nitrogen. The low amount and availability of protein can affect the amount of ammonia nitrogen in the rumen, compromising the fermentation dynamics performed by the microbiota (Satter and Slyter, 1974; Slyter et al., 1979). Ruminal ammonia concentration directly affects microbial growth and degradation of compounds in the rumen, which may affect consumption (Satter and Slyter, 1974; Slyter et al., 1979). Studies reported that DMI optimization
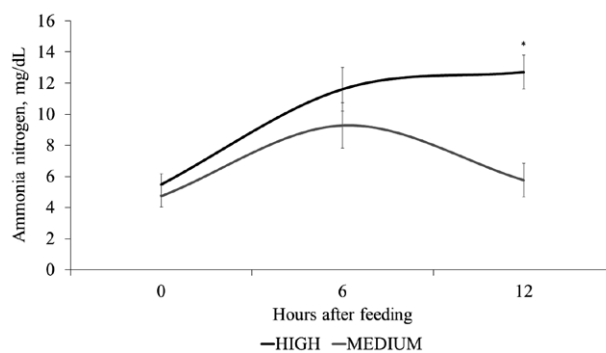
in animals fed tropical forages can occur with a ruminal ammonia concentration of 10 to 15 mg/dL (Ortiz-Rubio et al., 2007; Lazzarini et al., 2009). In the present study, the treatment that came close to these values was high-quality haylage, which indicates that the reduced DMI for the medium-quality haylage steers may also be associated with a reduced rumen ammoniacal nitrogen (Ortiz-Rubio et al., 2007; Lazzarini et al., 2009).

Multiple factors influence the proportion of ruminal SCFA, such as DMI, degradation rate, passage rate, and the absorption of acids by the ruminal epithelium during fermentation (Varel and Kreikemeier, 1999; Welkie et al., 2010; Alstrup et al., 2016; Wang et al., 2020). Also, the type of forage and the species and quantity of rumen bacteria influence the ratio of acetate, propionate, and butyrate produced in the rumen (Alstrup et al., 2016; Bharanidharan et al., 2018). Sampling time is also a valuable factor affecting ruminal SCFA production, which normally occurs after feeding when SCFA concentration significantly increases, and pH value drops in the rumen environment (Wang et al., 2020). Accordingly, in the current study, steers consuming high-quality forage increased the proportion of propionate, butyrate, isovalerate, and valerate, resulting in a reduced Ac:Prop and AcBut:Prop ratio after the feeding period.

In an effort to improve the productivity and profitability of forage-based beef cattle production systems, the adoption of nutritional tools, such as feed additives, is often recommended. By altering the microbial environment and fermentation dynamics in the rumen, as well as the efficient nutrient use and energy of the diet, feed additives are a crucial nutritional management tool (Tedeschi et al., 2003; Weimer et al., 2008; Schären et al., 2017; Marques and Cooke, 2021). Nonetheless, there are several feed additives commercially available for animal consumption that have similar effects on animal productivity, but their mechanisms in the rumen may vary depending on dosage, animal, frequency of use, and diet (Nagaraja et al., 1987; Tedeschi et al., 2003; Bretschneider et al., 2008; Limede et al., 2021; Soares et al., 2021). Additionally, there is scant or conflicting research about the effects of feed additives on intake, ruminal fermentative parameters, and performance when

offering a forage-based diet. Community researchers are interested in employing the ionophore narasin in a forage-based diet because of the possible advantage in performance and ruminal fermentation characteristics, with no direct impact on forage intake (Limede et al., 2021; Miszura et al., 2023), as normally observed in diets utilizing monensin (Duffield et al., 2012; Polizel et al., 2021b). Polizel et al. (2021c) observed that including 13 and 20 mg/kg of DM of narasin linearly increased the intake of lambs fed a high-quality haylage (CP = 148 g/kg of DM; NDF = 586 g/kg of DM). Additionally, the authors evaluated the interaction between forage quality and narasin and reported that narasin increased nutrient intake regardless of forage quality (Polizel et al., 2021c). In the present study, however, including 13 mg/kg of DM of narasin did not improve nutrient intake and digestibility of steers fed different haylage quality. Accordingly, Limede et al. (2021) and Miszura et al. (2023) reported no improvement in nutrient intake and digestibility of Nellore steers consuming narasin and haylage with similar quality used herein. Spears (1990) reported that the effects of ionophores on total tract digestibility might be associated with the type of diet consumed by the animals, with an absence of effects in forage-based diets. The present study is the first manuscript proposing to evaluate the interaction between forage quality and narasin inclusion on nutrient intake in Nellore steers, supporting that this additive could be used as a nutritional tool in forage-based systems without impairing animal intake.

It is well known that the inclusion of feed additives in concentrate- (Tedeschi et al., 2003; Duffield et al., 2012; Ellis et al., 2012) and forage-based (Bretschneider et al., 2008; Limede et al., 2021; Soares et al., 2021) diets alter ruminal fermentation toward propionate and decrease acetate concentration, which is positively correlated with greater feed energy utilization and performance (McGuffey et al., 2001; Weimer et al., 2008). Specifically, narasin supplementation improved the molar concentration of propionate by 3.6% and decreased the Ac:Prop and AcBut:Prop ratio by 4.1% and 4.2%, respectively, compared with the control cohorts, regardless of forage quality. The literature demonstrates that including narasin efficiently increases propionate in steers fed with forage-based diets, and the magnitude of this increase varies from 5.5% to 14% (Polizel et al., 2020; Limede et al., 2021), which corroborates with the results reported herein. Additionally, ionophores play an important role in the nitrogen metabolism of ruminant animals, with reports involving a reduction in protein degradation (Whetstone et al., 1981) and in the process of amino acid deamination (Chen and Russell, 1991), resulting in less accumulation of ammonia in ruminal fluid (Whetstone et al., 1981) and decrease in blood urea (Polizel et al., 2020). This reduction in ammonia could also be explained by the improvement in the energy efficiency of the fermentation process, resulting in greater use of ammonia by microorganisms (Polizel et al., 2021a). The results of the present investigation indicate that the degree to which narasin affects the ammonia concentration depends on the quality and protein content of the forage, with a decrease in concentration observed in diets with a higher CP content.

Collectively, this experiment provides information that narasin can increase ruminal fermentation efficiency for medium and high-quality forage, suggesting that it could be adopted as a feed additive in grazing systems, even with compositional variations of the forage. Furthermore, forage quality greatly influenced factors related to intake, digestibility, and ruminal fermentation.

Conflict of interest statement

None declared.

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