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Association of additives in supplemented grazing cattle during the finishing phase at the rainy season

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ABSTRACT - The objectives were to evaluate the effects of monensin and virginiamycin, alone or combined, on supplemented Nellore cattle grazing tropical grass during the rainy season. Two experiments were conducted simultaneously to evaluate intake, digestibility, CH_4 emissions, blood parameters, performance, and carcass characteristics (Exp. 1), and ruminal fermentation and relative abundance of ruminal microorganisms (Exp. 2). Animals (n = 92 Exp. 1 and n = 12 Exp. 2) were distributed in a completely randomized design and allocated in twelve paddocks composed of *Urochloa brizantha* (A. Rich.) Stapf. cv. Xaraés. A protein-energetic supplementation of 3 g/kg of BW per day was provided to all animals. Supplements were: without additives (WA), monensin alone at 80 mg/kg of product (MN), virginiamycin alone at 150 mg/kg of product (VM), and monensin (80 mg/kg of product) combined with virginiamycin (150 mg/kg of product; MNVM). Treatments did not affect intakes of total dry matter (DM), supplement DM, and nutrients. However, the intakes of forage DM and crude protein decreased in cattle fed MNVM compared with animals fed WA, MN, and VM. Total volatile fatty acids increased in animals fed VM. Ruminal NH_3-N decreased, and pH increased in animals fed MN, VM, and MNVM. Relative abundance of total *F. succinogenes* and *S. ruminantium* decreased and *R. flavefaciens* increased in animals fed MN and VM at d 118. Treatments had no effect on enteric CH_4 emissions. The average daily gain (ADG) and total gain were greater in cattle fed MNVM than in cattle fed MN. Combination of monensin and virginiamycin altered the rumen microbial populations but did not decrease enteric CH_4 emissions. However, it decreased forage dry matter intake without altering the ADG and total weight gain, leading to an increase in feed efficiency. Results from this study indicate an advantage in including feed additives combined in the diet of supplemented Nellore cattle grazing tropical grass during the rainy season.

Keywords: meat quality, methane, monensin, pasture, performance, virginiamycin

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1. Introduction

Forage supplementation as a strategy to improve the efficiency of nutrient utilization by microbiota is frequently required by ruminant nutritionists. Antibiotics feed additives have been successfully used in supplementation with concentrate (Bretschneider et al., 2008; Carvalho et al., 2017) and in

supplements to enhance rumen health, feed efficiency, and weight gain of animals in grazing systems (Tedeschi et al., 2003).

Ionophores such as monensin are known to increase propionate production and decrease the volatile fatty acids acetate and butyrate (Linneen et al., 2015). Additionally, this feed additive can reduce methane emission (Fonseca et al., 2016) and ruminal protein degradation, which results in less ammonia losses (Yang and Russell, 1993). However, animal performance results are controversial, in which an increase in gain was observed in feedlot cattle (Neumann et al., 2018). No changes in efficiency of metabolizable energy utilization for weight was reported in cattle fed tropical forages (Fonseca et al., 2016; Carvalho et al., 2017).

Virginiamycin, which is derived from *Streptomyces virginiae*, has been used in cattle feeding as a growth promoter. This non-ionophore antibiotic is known to inhibit the synthesis of peptides, improve the post-ruminal nutrient absorption, reduce the risk of lactic acidosis, and decrease energy loss in the form of gases (Owens et al., 1998). However, in the last decade, there has been an increasing search by consumers for beef produced without antibiotic utilization. In 2006, the EU banned the use of antibiotics, including virginiamycin in animal feed (Castagnino et al., 2018). However, Neumann et al. (2018) observed that the use of monensin for young bulls in confinement did not leave residues in edible tissues. These feed additives are known to maximize the symbiotic relationship of the microorganisms in the rumen, increase performance, and reduce methane emission in feedlot. However, the mode of action in which the association of these products and their dosages impact the rumen microbiota and performance of cattle grazing tropical grass during the rainy season is not completely understood (Rogers et al., 1995; Salinas-Chavira et al., 2009; Nuñez et al., 2013).

Therefore, the objectives of this study were to evaluate the effects of feed additives (monensin and virginiamycin) fed alone or in combination on ruminal fermentation, ruminal microorganisms, enteric methane emission, performance, and carcass characteristics of finishing supplemented Nellore cattle grazing tropical grass during the rainy season. The hypothesis was that the combination of monensin and virginiamycin would enhance the effects of modulation of rumen microbial populations, improving nutrient utilization and performance, while decreasing enteric CH_4 emission of the animals.

2. Material and Methods

The protocol used in this experiment was in accordance with the Brazilian College of Animal Experimentation) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (protocol number 021119/11).

2.1. Animals and management

Two experiments were carried out simultaneously. Experiment 1 evaluated intake, digestibility, CH_4 emissions, blood parameters, performance, and carcass characteristics of the animals. Experiment 2 evaluated ruminal fermentation and relative abundance of ruminal microorganisms of the animals.

The experiment was conducted during the rainy season from December 2013 to May 2014. According to the international Köppen classification, the climate of the region is characterized as tropical type Aw with rainy summer and a relatively dry winter. During the experimental period, the average monthly precipitation was 60.23 mm, with an average maximum and minimum monthly temperature of 33.5 °C and 14.5 °C, respectively. The experimental period lasted 112 d and was divided into four 28-d periods. The grazing method was the continuous stocking with variable ("put and take") stocking rate (Allen et al., 2011). Regulator animals were used to maintain canopy height at 30 cm, and stocking rate was adjusted weekly.

2.2. Experiment 1

Ninety-two Nellore bulls averaging (mean±SD) 30 months old and 360±24.98 kg of initial body weight (BW) were used for determination of performance and carcass characteristics. Before the beginning

of the grazing period, the animals were weighed, identified, and subjected to endo- and ectoparasite treatments utilizing ivermectin (Ivomec Injetável, 200 mg/kg, Merial Brasil, Campinas, SP, Brazil).

Animals were fed a protein-energetic supplement (Table 1) to meet their maintenance and BW gain requirements, aiming for volatile fatty acids (VFA) of 1.00 kg/day according to the Brazilian Nutrient Requirements for Zebu Beef Cattle system (Valadares Filho et al., 2016). The animals were subjected to four treatments: supplement without additives – WA; supplement with monensin inclusion (80 mg/kg product) – MN; supplement with virginiamycin inclusion (150 mg/kg of product) – VM; and supplement with monensin (80 mg/kg of product) in combination with virginiamycin (150 mg/kg of product) – MNVM.

Animals were distributed in a completely randomized design into 12 paddocks (considered the experimental unit) composed of *Urochloa brizantha* (A. Rich.) Stapf. cv. Xaraés pasture with three paddocks per treatment. Eleven paddocks of 1.8-ha each received eight animals per paddock and one of 1.0-ha received four animals. The animals were supplemented at 300 g/100 kg of BW daily at 10:00 h in collective covered feed bunks in each paddock and had free access to water.

After 15 days of adaptation to the diets, eight animals (379.13±51.65 kg) were slaughtered, serving as reference group to obtain carcass yield. The observed carcass yield was 54.72%, from which the initial carcass weight (CWi) of the remaining animals was estimated, aiming to obtain the carcass gain (CG) and CG in relation to the average daily gain (CG/ADG) at the end of the experiment.

Table 1 - Ingredients and chemical composition of supplements and pasture

Item	Treatment[1]				*Urochloa brizantha* cv. Xaraés[2]
	WA	MN	VM	MNVM	
Ingredient composition (g/kg DM)					
Citrus pulp	561.1	561.1	561.1	561.1	-
Cotton meal (38%)	313.2	313.2	313.2	313.2	-
Urea	34.9	34.9	34.9	34.9	-
Mineral mix[3]	51.8	51.8	51.8	51.8	-
Salt	38.6	38.6	38.6	38.6	-
Monensin (mg/kg)	-	80	-	80	-
Virginiamycin (mg/kg)	-	-	150	150	-
Chemical composition (g/kg DM)					
DM	929.3	927.6	925.2	928.6	330.5±4.6
Ash	231.2	220.3	202.1	215.2	74.4±5.9
CP	312.1	326.7	337.8	311.3	101.2±6.1
NDF	162.3	165.5	173.2	162.2	585.6±23.5
EE	16.7	15.8	17.8	17.6	14.2±2.2
NFC	277.6	271.7	269.2	293.8	224.6±37.7
GE (cal/g)	3161	3141	3454	3435	41694.2±614.9

DM - dry matter; CP - crude protein; aNDF-NDF - neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; EE - ether extract; NFC - non-fiber carbohydrates, calculated as 100 – (CP + EE + Ash + NDF); GE - gross energy (calculated according to Fiorentini et al., 2013).
[1] WA - supplement without additives; MN - supplement with monensin inclusion; VM - supplement with virginiamycin inclusion; MNVM - supplement with inclusion of monensin in combination with virginiamycin.
[2] Mean and standard deviation. Data from the simulated grazing technique in the four periods.
[3] Provided (per kg of DM): 210 g of Ca, 20 g of P, 37 g of S, 80 g of Na, 490 mg of Cu, 1,424 mg of Mn, 1,830 mg of Zn, 36 mg of I, 29 mg of Co, 9 mg of Se, and 333 mg of F (maximum).

2.2.1. Herbage sampling

Grazing height was measured weekly at 80 random points per hectare (Barthram, 1985). Herbage mass was estimated using four samples per paddock, cut at the ground level (5 cm residual height), from average pasture height points of the paddock, using a frame of 0.25 m^2 area, every 28 days

(January to April 2013). Samples were dried at 55 °C to constant weight to estimate DM/ha. Herbage samples used for chemical analyses were hand-plucked in the same periods in 20 average spots heights at each paddock, dried at 55±5 °C to constant weight and ground through a 1-mm screen in a shear mill (Thomas Wiley Laboratory Mill Model 4, H. Thomas Co.).

2.2.2. Chemical analyses

Dry matter (DM; 934.01) and organic matter (OM; 942.05) were determined according to procedures from AOAC (1990). Crude protein (CP) was determined using LECO® FP 528 (Leco Corporation, MI, USA). Neutral detergent fiber (NDF) was determined by adding alpha-amylase and expressed inclusive of residual ash (aND-NDF) according to Mertens (2002) with adaptations for ANKOM® Fiber Analyzer (Ankom technologies, NY, USA). Gross energy (GE) was determined using adiabatic bomb calorimeter (PARR Instrument Company 6300, IL, USA).

2.2.3. Intake estimation

From the 92 animals used for performance evaluation, 32 (n = 8 per treatment) were used for feed intake determinations, which was performed starting from the 118th day of the experimental period. Two markers were used to determine the fecal production and pasture intake. Supplement intake was measured in relation to that provided in the paddocks.

The fecal production was determined using the external maker chromium oxide (Cr_2O_3) for 10 days, administering 12 g/animal/day by using a rubber tube directly into the esophagus at the time of supplementation (10.00 h), for 7 d to stabilize fecal excretion of the marker and 3 d for sample collection. Fecal samples were collected directly from the rectum of each animal, in three different times during the day (07:00, 10:00, and 17:00 h) removing approximately 100 to 200 g of feces per sampling time.

After collected, the samples were immediately frozen and stored for future analysis. Then, fecal samples were thawed and dried in a forced ventilation oven at 55 °C until constant weight for the determination of DM. Subsequently, samples were ground (Wiley mill; Thomas Scientific) through a 1-mm mesh. The concentration of Cr_2O_3 in the fecal samples was determined by atomic absorption spectrophotometry as described by Williams et al. (1962).

Fecal excretion was estimated using the following equation:

$$\text{Fecal excretion} = Cr_2O_3 \text{ administered (g/d)} - Cr_2O_3 \text{ concentration in feces (g/g DM)}$$

Chromium oxide recovery rates (CRr) were calculated through the total chromium excreted as follows:

$$\text{CRr} = \text{fecal } Cr_2O_3 \text{ (g/kg)} \times \text{kg feces}/Cr_2O_3 \text{ administered (g)}$$

The individual forage dry matter intake (DMI) was estimated using the internal marker indigestible neutral detergent fiber (iNDF). Feces, forage, and concentrate samples were placed in ANKOM bags (filter bag F57; ANKOM Technology Corp.) and incubated in the rumen of four cannulated Nellore animals for a period of 288 h (Valente et al., 2011). After that, the bags were removed from the rumen, soaked in water for 30 min, and gently hand-washed under running water until the wash water was clear. Then, bags were analyzed for aNDF-NDF concentration using an Ankom^{200} Fiber Analyzer (Ankom Technology, Fairport, NY, USA). The iNDF concentration in the samples was determined by weighing the bags after drying in an oven, first at 55 °C for 72 h, followed by 105 °C for 12 h. The residue was considered the iNDF content. Individual forage DMI was estimated by subtracting the marker of supplement from the total iNDF excretion and dividing that difference by the concentration of the marker in the forage.

Individual supplement DMI was estimated by dividing the total supplement provided by the number of animals in each paddock.

2.2.4. Enteric methane emissions

From the 92 animals used for performance evaluation, 32 (n = 8 per treatment) were used for enteric CH_4 emissions determinations, which was performed on the same days used for feed intake estimation. For that, the sulfur hexafluoride (SF_6) tracer method was used according to Johnson et al. (1994). Capsules with constant release of SF_6 were inserted orally into the rumen of the animals. The sampling apparatus consisted of a polyvinyl chloride collection vessel and a capillary tube extending from the collection canister to just above the mouth and nostrils of the animals. The canister was attached to a collar placed around the neck of the animal. Additional identical set of canisters (two per day) were placed near the experimental pasture to collect background (environmental) concentration of CH_4 and SF_6 at the same time canisters were collected from the animals.

Before the beginning of the sample collection, the attached canister was connected to the transfer line and a valve on the collection vessel was opened. The collection vessel was changed daily during six consecutive days. Concentrations of CH_4 and SF_6 were analyzed by a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a column Porapak Q (2 m × 3 mm i.d., 80 to 100 mesh, Shimadzu, Kyoto, Japan), flame ionization detector for CH_4, and electron capture detector for determination of SF_6 concentration. Animal enteric CH_4 emission was calculated in proportion to SF_6 capsule emission in the rumen, subtracting the environmental CH_4 concentration as follows:

$$CH_4 = CSF_6 \times ([CH_4]v - [CH_4]_{En}) / [SF_6]v,$$

in which CH_4 is the animal CH_4 daily emission rate, CSF_6 is the known SF_6 emission from the capsule in the rumen, $[CH_4]v$ is the CH_4 concentration at collection vessel, $[CH_4]_{En}$ is the CH_4 concentration in the environment (background), and $[SF_6]v$ is the SF_6 concentration at collection vessel. Enteric CH_4 emission was expressed as g CH_4/day, kg CH_4/year, g CH_4/kg DMI, g CH_4/kg NDFi, g CH_4/kg GEi, g CH_4/kg BWG and g CH_4/kg of CG.

2.2.5. Blood parameters

Jugular vein blood samples were collected from all animals after 16 h of solid fast and before the morning feeding at days 0, 63, and 118. Blood samples were collected in Vacutainer tubes (10 mL; BD Biosciences, Franklin Lakes, NJ) and EDTA-coated glass Vacutainer tubes (10 mL; BD Biosciences, Franklin Lakes, NJ). The tubes were immediately placed on ice and centrifuged at 2500 × g for 20 min at 4 °C. The resulting serum or plasma was collected and stored at −20 °C until laboratory analysis. Fasting plasma samples were analyzed for glucose concentration (Glucose Liquiform Vet Kit, Labtest Diagnostica S.A., Lagoa Santa, Brazil), and fasting serum samples were analyzed for insulin concentration (ADVIA Centaur CP Insulina – IRI, manufactured in Japan by Kyowa Medex Co., Ltd. for Siemens Healthcare Diagnostics Inc., Tarrytown, New York, USA) using commercial kits.

2.2.6. Animal performance

The experimental period was 112 days, and the animals were weighed at the beginning and end of the experiment after a 14-h fasting period. Performance parameters were calculated using the equations:

$$BWG \text{ (body weight gain)} = \text{final BW (kg)} - \text{initial BW (kg)}$$

$$ADG = BWG \text{ (kg)}/112 \text{ (days)}$$

Additionally, the animals were weighed without fasting every 28 days for adjustment of the supplementation rate (% BW).

2.2.7. Slaughter procedure

At the end of the experimental period, the animals were transported to a slaughterhouse (Minerva, Barretos, São Paulo, Brazil), where they were slaughtered following the standard procedures. After

fasting (from feed) for 24 h, slaughter was performed using a compressed air pistol to cause a cerebral concussion, according to humanely slaughter under Brazilian federal inspection (Brasil, 2000).

After slaughter, the carcasses were identified, weighted, and refrigerated at 4 °C for approximately 24 h. Carcass yield was calculated based on the hot carcass weight (HCW) and BW ratio after fasting. After the postmortem chill period, the cold carcass weight (CCW), 12th rib fat thickness (RFT), and 12th rib *longissimus* muscle area (LMA) were measured on the left side of each carcass.

The LMA was traced on transparencies and measured later with a planimeter, and RFT measurements were taken at 3/4 of the length, ventrally over the *longissimus* muscle (Greiner et al., 2003). Cold carcass dressing percent (CCD) was calculated using CCW divided by final shrunk body weight (SBW) and then multiplying the result by 100.

2.3. Experiment 2

Twelve Nellore steers cannulated in the rumen were allocated in 12 paddocks (one animal per paddock), arranged in a completely randomized design, totalizing three animals per treatment, in four periods of 28 days each. This design was chosen to observe the short- and long-term effects of the use of monensin and virginiamycin on a microbial population.

2.3.1. Ruminal fermentation

Sampling of ruminal material was performed every 28 days, with 27 days for adaptation and one day for collection. To determine VFA, aliquots of 50 mL of ruminal contents were obtained at 0, 3, 6, 9, and 12 h after supplementation (10:00 h), from several sites within the rumen. Then, the samples were strained through two layers of cheesecloth and centrifuged at 13,000 × *g* (4 °C) for 30 min. The VFA were quantified by gas chromatography, using a GC2014 (Shimatzu Corporation, Kyoto, Japan), with an HP-INNOWax capillary column (30 m × 0.32 mm; 0.50-µm film thickness; Agilent Technologies, CO) at an initial temperature of 80 °C and a final temperature of 240 °C.

2.3.2. Rumen microbial analysis

Samples (70 g) of rumen content (solid + liquid) were collected at day 28 of each experimental period (before the morning feeding). Then, they were immediately mixed with PBS buffer (1% Tween, pH 7.4), processed to obtain a microbial pellet according to Granja-Salcedo et al. (2017) and frozen at −20 °C until DNA extraction. A sample of 200 mg of the bacterial pellet was used for DNA extraction using "Fast spin kit for soil" from MP Bio® according to manufacturer's instructions, and the FastPrep-24 Classic Instrument (MP Bio, Biomedicals, Illkirch, France) to lyse cells. Yield and quality of DNA were evaluated by spectrophotometry (NanoDrop 1000, Thermo Fisher Scientific, Waltham, MA, USA) and by fluorometry (Qubit 3.0, Life Technology, Waltham, MA, USA). The integrity of DNA was verified on a 0.8% agarose gel stained with ethidium bromide (5 mg/mL).

The amplifications were performed in triplicates, and negative controls were used in the assay, omitting the total DNA. Real-time PCR was performed with Applied Biosystems 7500 Real-time PCR System (Applied Biosystems). Rox was used as a passive reference dye. Four concentrations (200, 400, 600, and 800 nM) of forward and reverse primers were tested to determine minimum primer concentration giving the lowest threshold cycle (Ct) and to reduce nonspecific amplification before starting the reaction. The slope value and the efficiency of selected-primers concentrations were calculated with different DNA concentrations (150, 75, 37.5, 18.75, and 9.37 ng).

The primer sets used for qPCR are described in Table 2. Conditions for PCR were 50 °C for 2 min, 95 °C for 10 min, 35 cycles of 95 °C for 15 s, and 60 °C for 1 min. Each conventional PCR mixture (12.5 µL) contained (final concentrations) 1× Power SYBR Green PCR Master Mix (Applied Biosystems), 400 or 600 nM of each primer, and 150 ng of metagenomic DNA and ultrapure water. Specificity of amplified products was confirmed by melting temperatures and dissociation curves after each amplification.

Amplicon specificity was performed via dissociation curve analysis of PCR end products. Relative quantification was used to determine species proportion. The results were expressed as a 16S rDNA ratio of general bacteria (Denman and McSweeney, 2006), following the equation:

$$\text{Relative quantification} = 2- (\text{Ct target} - \text{Ct total bacteria}),$$

in which Ct is defined as the number of cycles required for the fluorescent signal to cross the threshold. The relative abundance was adjusted by the primer efficiency correction according to (Pfaffl et al., 2002).

Table 2 - Target primers used in the relative quantification of ruminal bacteria by qPCR analysis

Primer	Sequence (5' to 3')	Bp	Efficiency (%)
Total bacteria[1]	F: CGGCAACGACAACCC	130	101.50
	R: CCATTGTAGCACCTGTGTAGCC		
Fibrobacter succinogenes[2]	F: GTTCGG AATTAC TGG GCGTAAA	121	97.00
	R: CGCCTGCCCCTGAACTATC		
Ruminococcus albu[1]	F: CCCTAAAAGCAGTCTTAGTTCG	175	100.50
	R: CCTCCTTGCGGTTAGAACA		
Ruminococcus flavefaciens[1]	F: CGAACGGAGATAATTTGAGTTTACTTAGG	132	98.00
	R: CGGTCTCTGTATGTTATGAGGTATTACC		
Selenomonas ruminantium[3]	F: GGCGGGAAGGCAAGTCAGTC	83	97.50
	R: CCTCTCCTGCACTCAAGAAAGACAG		
Total Archaea[4]	F: TTCGGTGGATCDCARAGRGC	140	100.50
	R: GBARGTCGWAWCCGTAGAATCC		

F - forward; R - reverse; BP - amplicon size of base pairs.
[1] Primers were set from Denman and McSweeney (2006).
[2] Primers were set from Koike and Kobayashi (2001).
[3] Primers were set from Khafipour et al. (2009).
[4] Primers were set from Denman et al. (2007).

2.4. Statistical analysis

Statistical analyses were performed using R Software version 3.5.1 (R Core Team, 2015), and the data were initially tested for the mathematical assumptions with Shapiro–Wilk test and Bartlett tests. The statistical model used was:

$$Y_{ijkl} = \mu + b_i + MN_j + VG_k + (MN \times VG)_{jk} + e_{ijk},$$

in which Y_{ijkl} represents the observation on experimental unit *l* supplemented with monensin inclusion (with and without) *j* and virginiamycin inclusion (with or without) *k* in block *i*; μ = the overall mean; b_i = the block effect *i*; MN_j = factor 1 corresponding to monensin inclusion (with and without) *j*; VG_k = factor 2 corresponding to virginiamycin inclusion (with or without) *k*; $MN_j \times VG_k$ = factor interactions *jk*; and e_{ijk} = the residues corresponding to each observation.

For Experiment 1, the data of intake, digestibility, methane emissions, performance, and carcass characteristics were compared between treatments by ANOVA as randomized block design in a double factorial arrangement (A×B) considering the paddock as the experimental unit. The fixed effects considered were factor A, corresponding to monensin inclusion (with and without), and factor B, corresponding to the virginiamycin inclusion (with or without), factors interactions, block, treatments error, and the random effects of residues corresponding to the model.

Blood parameters data from Experiment 1 and pH, NH_3-N, and VFA from Experiment 2 were compared among treatments and time as repeated-measures using ANOVA in a completely randomized design in a split-plot factorial arrangement (A×B) considering the animal as the experimental unit. The fixed

effects considered were factor A, monensin inclusion (with and without), and factor B, virginiamycin inclusion (with and without), that were considered as independent variables; sampling time (covariate), interactions, and treatments residues were considered as random effects. The random effects were periods and residues error corresponding to the model. Tukey's post hoc test was applied when ANOVA indicated a significant difference, considering statistical significance when P≤0.05.

Data of relative abundance of bacteria and Archaea were compared between sampling day, and the use of monensin or virginiamycin using a Friedman's test, and the interaction by Kruskal-Wallis and Dunn's post-hoc test.

3. Results

3.1. Experiment 1

3.1.1. Intake and digestibility

The inclusion of feed additives MN, VM, and MNVM in the diet of supplemented finishing Nellore cattle grazing tropical grass in the rainy season did not affect (P>0.05) the intakes of total DM, supplement DM, OM, NDF, and GE (Table 3). Similarly, treatments had no effect on the apparent digestibility of DM, OM, CP, NDF, and GE (P>0.05). However, an interaction between MN and VM was observed for the intakes of forage DM (P<0.033) and CP (P<0.022), which decreased in animals fed MNVM (Table 3).

Table 3 - Effects of feed additives alone or in combination on intake and apparent digestibility of supplemented finishing Nellore cattle grazing tropical grass in the rainy season (Experiment 1)

Item	Treatment[1]				SEM	P-value[2]		
	WA	MN	VM	MNVM		M	V	M×V
Intake (kg/d)								
DM	12.19	11.78	11.75	10.41	0.752	0.092	0.076	0.350
Forage DM	10.70a	10.32a	10.29a	8.94b	0.750	0.094	0.079	0.033
Supplement DM	1.49	1.47	1.46	1.46	0.009	0.486	0.070	0.195
OM	11.05	10.69	10.68	9.42	0.482	0.092	0.085	0.328
CP	1.49a	1.53a	1.60a	1.40b	0.054	0.145	0.876	0.022
NDF	6.40	6.16	6.13	5.30	0.308	0.082	0.064	0.317
GE (Mcal/kg)	4.97	4.75	4.74	4.24	0.216	0.095	0.083	0.488
Digestibility (% of DM)								
DM	61.57	61.75	61.58	62.32	1.412	0.732	0.827	0.834
OM	63.80	63.42	63.49	62.68	1.286	0.628	0.664	0.860
CP	73.13	74.77	75.55	76.22	1.377	0.359	0.146	0.697
NDF	58.67	57.94	55.93	58.55	1.546	0.525	0.469	0.259
GE	69.19	68.84	69.03	69.16	1.187	0.922	0.944	0.832

DM - dry matter; OM - organic matter; CP - crude protein; aNDF-NDF - neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; GE - gross energy (calculated according to Fiorentini et al., 2013); SEM - standard error of the mean.
[1] WA - supplement without additives; MN - supplement with monensin inclusion; VM - supplement with virginiamycin inclusion; MNVM - supplement with inclusion of monensin in combination with virginiamycin.
[2] M - inclusion of monensin alone; V - inclusion of virginiamycin alone; M×V - interaction between monensin and virginiamycin inclusion.
a-b - Least squares means within the same row with different letters are significantly different (P<0.05).

3.1.2. Enteric methane emission

Enteric methane emissions (g/day, kg/year, g/kg of DM intake, g/kg of NDF intake, g/kg of GE intake, g/kg of BWG, and g/kg of CG) of supplemented finishing Nellore cattle grazing tropical grass in the rainy season were not affected (P>0.05) by the inclusion of feed additives (Table 4).

Table 4 - Effects of feed additives alone or in combination on enteric methane (CH_4) emission of supplemented finishing Nellore cattle grazing tropical grass in the rainy season (Experiment 1)

Item	Treatment[1]				SEM	P-value[2]		
	WA	MN	VM	MNVM		M	V	M×V
CH_4 (g/d)	112.9	111.5	109.7	116.6	3.458	0.441	0.787	0.245
CH_4 (kg/year)	41.22	40.70	40.05	42.56	1.262	0.441	0.788	0.244
CH_4 (g/kg DMI)	9.57	8.28	9.81	9.78	0.658	0.351	0.204	0.332
CH_4 (g/kg NDFI)	17.70	17.15	18.87	19.23	1.177	0.927	0.172	0.686
CH_4 (g/kg GEI)	4.26	4.37	4.23	4.01	0.253	0.845	0.422	0.497
CH_4 (g/kg BWG)	120.1	130.6	116.6	113.3	7.059	0.613	0.158	0.339
CH_4 (g/kg CG)	265.1	295.0	247.8	244.6	21.68	0.571	0.161	0.481

DMI - dry matter intake; NDFI - neutral detergent fiber intake; GEI - gross energy intake; BWG - body weight gain; CG - carcass gain; SEM - standard error of the mean.
[1] WA - supplement without additives; MN - supplement with monensin inclusion; VM - supplement with virginiamycin inclusion; MNVM - supplement with inclusion of monensin in combination with virginiamycin.
[2] M - inclusion of monensin alone; V - inclusion of virginiamycin alone; M×V - interaction between monensin and virginiamycin inclusion.

3.1.3. Blood parameters

The inclusion of MN, VM, and MNVM in the diet of supplemented finishing Nellore cattle grazing tropical grass in the rainy season did not change (P>0.05) the blood glucose concentration (g/L) of the animals (Table 5). However, it was affected by sampling day (P<0.001), in which the greatest blood glucose concentration was observed at day 118 (mean 0.86 g/L) and the lowest at day 63 (mean 0.32 g/L). Blood insulin concentration (µmol/L) was increased (P = 0.037) in treatments with MN inclusion compared with treatments with VM inclusion (87.71 vs. 79.92 µmol/L, respectively; Table 5).

Table 5 - Effects of feed additives alone or in combination on blood parameters of supplemented finishing Nellore cattle grazing tropical grass in the rainy season (Experiment 1)

Item	Treatment[1]				SEM	P-value[2]						
	WA	MN	VM	MNVM		d	M	V	M×d	V×d	M×V	M×V×d
Glucose (g/L)	0.63	0.59	0.60	0.58	0.04	<0.001	0.351	0.168	0.308	0.742	0.814	0.898
Insulin (µmol/L)	79.27	85.94	80.58	89.47	2.77	0.232	0.037	0.129	0.450	0.123	0.924	0.881

SEM - standard error of the mean.
[1] WA - supplement without additives; MN - supplement with monensin inclusion; VM - supplement with virginiamycin inclusion; MNVM - supplement with inclusion of monensin in combination with virginiamycin.
[2] d - sampling day; M - inclusion of monensin alone; V - inclusion of virginiamycin alone; M×d - interaction between monensin inclusion and sampling day; V×d - interaction between virginiamycin inclusion and sampling day; M×V - interaction between monensin and virginiamycin inclusion; M×V×d - interaction between monensin and virginiamycin inclusion and sampling day.

3.1.4. Performance and carcass characteristics

The initial BW, final BW, CG/ADG, HCW, HCD, CCW, and RFT of supplemented finishing Nellore cattle grazing tropical grass in the rainy season were not affected (P>0.05) by the inclusion of feed additives (Table 6). The ADG (kg/d) and total weight gain (kg) decreased in animals fed MN compared with the those fed the WA, VM, and MNVM treatments (P>0.05).

An interaction (P<0.001) between monensin and virginiamycin was observed for feed efficiency with greatest results presented by animals fed MNVM compared with animals fed the other treatments (Table 6).

Table 6 - Effects of feed additives alone or in combination on performance and carcass characteristics of supplemented finishing Nellore cattle grazing tropical grass in the rainy season (Experiment 1)

Item	Treatment[1]				SEM	P-value[2]		
	WA	MN	VM	MNVM		M	V	M×V
Initial BW (kg)	365.95	370.76	373.55	368.47	5.588	0.981	0.641	0.603
Final BW (kg)	479.80	471.40	475.20	483.00	6.914	0.486	0.812	0.330
ADG (kg/d)	0.96a	0.83b	0.90a	0.98a	0.028	0.301	0.144	<0.001
Total gain (kg)	113.80a	97.98b	106.60a	115.30a	5.261	0.300	0.144	<0.001
CG/ADG	45.89	47.89	46.59	46.41	1.315	0.503	0.776	0.425
HCW (kg)	256.30	258.1	255.9	259.6	4.052	0.497	0.896	0.815
HCD (%)	53.42	54.61	54.05	53.95	0.323	0.101	0.982	0.053
CCW (kg)	252.2	253.3	250.9	257.1	3.896	0.366	0.759	0.528
CCD (%)	52.57	53.75	53.17	53.19	0.288	0.047	0.956	0.056
RFT (mm)	2.16	2.33	2.23	2.19	0.196	0.734	0.846	0.599
FE	0.078b	0.071b	0.076b	0.094a	0.007	0.172	0.190	<0.001

BW - body weight; ADG - average daily gain; CG - carcass gain; HCW - hot carcass weight; HCD - hot carcass dressing; CCW - cold carcass weight; CCD - cold carcass dressing; RFT - rib fat thickness; FE - feed efficiency; SEM - standard error of the mean.
[1] WA - supplement without additives; MN - supplement with monensin inclusion; VM - supplement with virginiamycin inclusion; MNVM - supplement with inclusion of monensin in combination with virginiamycin.
[2] M - inclusion of monensin alone; V - inclusion of virginiamycin alone; M×V - interaction between monensin and virginiamycin inclusion.
a-b - Least squares means within the same row with different letters are significantly different (P<0.05).

3.2. Experiment 2

3.2.1. Ruminal fermentation

The inclusion of feed additives MN, VM, and MNVM in the diet of supplemented finishing Nellore cattle grazing tropical grass in the rainy season did not affect (P>0.05) the molar proportion of acetate, propionate, butyrate, isovalerate, valerate, and the acetate:propionate ratio (Table 7). However, an interaction (P<0.001) between MN and VM was observed for rumen pH, which increased in animals fed MNVM (Table 7).

Table 7 - Effects of feed additives alone or in combination on ruminal fermentation of supplemented finishing Nellore cattle grazing tropical grass in the rainy season (Experiment 2)

Item	Treatment[1]				SEM	P-value[2]						
	WA	MN	VM	MNVM		T	M	V	M×T	V×T	M×V	M×V×T
pH	6.25b	6.49a	6.55a	6.57a	0.03	<0.001	0.005	<0.001	0.911	0.653	<0.001	0.988
NH_3-N (mg/dL)	19.92	17.72	18.49	16.27	0.95	<0.001	0.021	0.032	0.978	0.343	0.562	0.798
Total VFA (mmol/L)	117.63	118.10	120.98	118.74	3.10	0.013	0.795	0.464	0.684	0.778	0.618	0.395
VFA (% of total VFA)												
Acetate	72.12	71.65	71.47	72.04	0.34	0.153	0.892	0.730	0.199	0.394	0.131	0.927
Propionate	16.82	16.88	17.08	16.64	0.17	0.115	0.217	0.973	0.132	0.147	0.198	0.561
Isobutyrate	0.68	0.68	0.74	0.70	0.02	0.049	0.241	0.017	0.862	0.687	0.275	0.530
Butyrate	9.02	9.06	8.53	8.95	0.18	0.377	0.288	0.092	0.597	0.922	0.286	0.833
Isovalerate	1.00	0.98	0.92	0.93	0.03	0.408	0.828	0.058	0.684	0.854	0.617	0.918
Valerate	0.86	0.89	0.91	0.90	0.04	0.226	0.827	0.556	0.243	0.676	0.697	0.968
A:P	4.33	4.28	4.23	4.34	0.07	0.367	0.641	0.744	0.260	0.380	0.264	0.724

NH_3-N - ammonia nitrogen; VFA - volatile fatty acids; A:P: acetate to propionate ratio; SEM - standard error of the mean.
[1] WA - supplement without additives; MN - supplement with monensin inclusion; VM - supplement with virginiamycin inclusion; MNVM - supplement with inclusion of monensin in combination with virginiamycin.
[2] T - sampling time; M - inclusion of monensin alone; V - inclusion of virginiamycin alone; M×T - interaction between monensin inclusion and sampling time; V×T - interaction between virginiamycin inclusion and sampling time; M×V - interaction between monensin and virginiamycin inclusion; M×V×T - interaction between monensin and virginiamycin inclusion and sampling time.
a-b - Least squares means within the same row with different letters are significantly different (P<0.05).

An effect of sampling time was observed for rumen pH (P<0.001), ruminal NH_3-N concentration (P<0.001), total VFA concentration (P = 0.013), and isobutyrate molar proportion (P = 0.049; Table 7). The lowest value of rumen pH was found at 12 h after supplementation compared with 0 and 3 h (6.29, 6.79, and 6.50, respectively). For ruminal NH_3-N concentration, the greatest value was observed at 3 h (30.09 mg/dL) after supplementation; and the lowest value of total VFA was observed at 3 h (104.52 mmol/L) after supplementation when compared with 0 (126.64 mmol/L), 9 (124.66 mmol/L), and 12 h (122.91 mmol/L). Ruminal NH_3-N concentration of supplemented finishing Nellore cattle grazing tropical grass in the rainy season decreased (P<0.05) in animals fed MN and VM. Furthermore, isobutyrate molar proportion was greater (P = 0.017) in animals fed VM (Table 7).

3.2.2. Ruminal microorganisms

Total Archaea and *Ruminococcus albus* relative abundance of supplemented finishing Nellore cattle grazing tropical grass in the rainy season were not affected (P>0.05) by sampling day and treatments (Table 8). However, sampling day altered the relative abundance of *Fibrobacter succinogenes* (P = 0.013), *Ruminococcus flavefaciens* (P = 0.007), and *Selenomonas ruminantium* (P = 0.002). The relative abundance of *Fibrobacter succinogenes* and *Selenomonas ruminantium* decreased in animals fed MN and VM at d 118 compared with d 28. In contrast, the relative abundance of *Ruminococcus flavefaciens* increased in animals fed MN and VM at d 118 (Table 8).

Table 8 - Effects of feed additives alone or in combination on the relative abundance (medians and interquartiles) of cellulolytic bacteria and methanogenic Archaea in the rumen of supplemented finishing Nellore cattle grazing tropical grass in the rainy season (Experiment 2)

Item[1]		Treatment[2]				P-value[3]			
	Day	WA	MN	VM	MNVM	d	M	V	M×V
Fibrobacter succinogenes	28	0.561±0.08	0.958±0.33	0.895±0.64	0.912±0.22	0.013	0.033	0.040	0.354
	118	0.613±0.12	0.688±0.23	0.795±0.11	0.744±0.09				
Ruminococcus albus	28	0.045±0.02	0.061±0.05	0.052±0.01	0.057±0.03	0.251	0.468	0.397	0.652
	118	0.053±0.01	0.049±0.04	0.066±0.02	0.051±0.04				
Ruminococcus flavefaciens	28	0.122±0.04	0.012±0.01	0.009±0.00	0.011±0.01	0.007	0.048	0.037	0.209
	118	0.178±0.03	0.133±0.01	0.083±0.01	0.097±0.02				
Selenomonas ruminantium	28	0.009±0.03	0.058±0.02	0.049±0.01	0.054±0.03	0.002	0.001	0.001	0.318
	118	0.010±0.02	0.021±0.03	0.019±0.02	0.022±0.02				
Total Archaea	28	1.785±0.23	1.591±0.18	1.433±0.11	1.338±0.14	0.218	0.115	0.096	0.129
	118	1.505±0.32	1.698±0.25	1.707±0.09	1.596±0.28				

[1] Measured based on the proportion of the specific 16S rRNA associated with total bacteria.
[2] WA - supplement without additives; MN - supplement with monensin inclusion; VM - supplement with virginiamycin inclusion; MNVM - supplement with inclusion of monensin in combination with virginiamycin.
[3] Obtained using Dunn's test; d - sampling day; M - inclusion of monensin alone; V - inclusion of virginiamycin alone; M×V - interaction between monensin and virginiamycin inclusion.

4. Discussion

Monensin has been widely studied since its discovery and is well recognized for improving feed efficiency, reducing DM intake, and increasing ADG of cattle (Goodrich et al., 1984; Duffield et al., 2012). Virginiamycin is a non-ionophore feed additive known to play an important role in the modulation of rumen fermentation. It can improve feed efficiency in cattle (Salinas-Chavira et al., 2009) by inhibiting ruminal bacteria growth through inhibition of their protein synthesis (Cocito, 1979; Nagaraja and Taylor, 1987). Nonetheless, contrary to our hypothesis, the combination of monensin and virginiamycin did not change digestibility, enteric CH_4 emission, and carcass characteristics of the animals.

In the present study, it was observed that animals fed monensin in combination with virginiamycin consumed 16% less forage DM and 5.78% less CP without altering the ADG compared with animals fed diet with no additive inclusion, which indicates an improvement in the feed efficiency of those animals. Corroborating our findings, in a meta-analysis evaluating the effects of monensin inclusion in beef cattle diets, Duffield et al. (2012) observed a decrease in DMI and improvement in feed efficiency and ADG in monensin-supplemented growing and finishing beef cattle. Goodrich et al. (1984) evaluating performance data of approximately 16,000 cattle, reported that animals fed monensin gained more weight and consumed less feed than animals fed control diets. Additionally, Oliveira et al. (2015) reported a decrease in 14% on pasture DMI when supplemented lactating cows on pasture received virginiamycin. Rogers et al. (1995) conducted a series of studies to evaluate the effects of virginiamycin on performance of feedlot cattle and observed an increase in ADG and feed conversion when animals were fed diets with the additive inclusion. However, Lemos et al. (2016) and Maciel et al. (2019) reported no benefits of the use of monensin in combination with virginiamycin on DMI and ADG of finishing zebu cattle fed a no-roughage whole shelled corn (WSC)-based diet.

The present study demonstrated that ruminal pH was directly affected by the inclusion of feed additives in the diet. We observed that ruminal pH of supplemented Nellore cattle grazing tropical grass in the rainy season increased in animals fed monensin and virginiamycin alone or in combination. Our findings corroborates other studies that reported that ionophores such as monensin can alter ruminal fermentation resulting in favorable metabolic changes in the rumen and moderate ruminal pH fluctuation (Nagaraja et al., 1982; Bergen and Bates, 1984). In addition, similarly to other ionophores, virginiamycin has been shown to play a role in the stabilization of ruminal fermentation and pH (Rogers et al., 1995). In an *in vitro* study evaluating the effects of monensin and essential oils supplementation on ruminal fermentation, Li et al. (2013) observed a tendency of monensin-containing diet to increase pH. In addition, Coe et al. (1999), evaluating the effects of virginiamycin on ruminal fermentation of cattle during an induced acidosis, reported greater ruminal pH on cattle receiving virginiamycin compared with controls.

It has been demonstrated by *in vivo* and *in vitro* studies that monensin can inhibit wasteful ruminal protein degradation (Dinius et al., 1976; Van Nevel and Demeyer, 1977), decrease the number of amino acid-fermenting bacteria (Yang and Russell, 1993) and the synthesis of ruminal NH_3, and increase ruminal bypass of feed-protein (Poos et al., 1979). Therefore, a decrease in ruminal NH_3 concentration would be expected in animals fed monensin due to the reduction in AA deamination. In line with those findings, the present study observed a decrease in ruminal NH_3-N concentration in animals fed monensin and virginiamycin compared with control animals. In addition, Coe et al. (1999), evaluating the effects of virginiamycin on ruminal fermentation of cattle during an induced acidosis, reported that ruminal NH_3 concentration was unaffected by the feed additive. Harmon et al. (1993) reported that NH_3-N portal flux was unchanged in steers receiving alfalfa hay and monensin supplementation. According to Detmann et al. (2009), ruminal NH_3-N concentration at 8 and 15 mg/dL optimize fiber degradation of low-quality tropical forage. In the present study, the mean value of NH_3-N across treatments was within that range (17.5 mg/dL) and may help explain the absence of difference in the digestibility of NDF and the other nutrients.

Although the inclusion of feed additives altered ruminal pH and NH_3-N concentration, the absence of changes in nutrient digestibility among treatments might have reflected in similar rumen fermentation parameters as observed for total VFA concentration and profile. Additionally, the lack of differences in the total VFA concentration and profile could help to explain similar animal performance and carcass characteristics across treatments. Similar results were reported by Lemos et al. (2016), who observed no differences in total VFA concentration and ADG of finishing zebu cattle fed a no-roughage WSC-based diet supplemented with monensin and virginiamycin alone or combined. Additionally, it is in line with several studies that have reported little or no effects of monensin supplementation on ruminal VFA molar proportion (Richardson et al., 1976; Givens et al., 1981; Galyean et al., 1992; Zinn et al., 1994).

As previously mentioned, ionophores can alter ruminal fermentation and cause favorable metabolic changes in the rumen (Bergen and Bates, 1984) such as increase in propionate synthesis and decrease

in CH_4 production (Chen and Wolin, 1979). Those alterations are commonly attributed to shifts in the microbial population of the rumen, especially on carbohydrate-fermenting bacteria and methanogenic archaea, which are known to be more sensitive to feed additives (Chen and Wolin, 1979). In line with that, the present study demonstrated that the inclusion of monensin and virginiamycin alone in the diet of supplemented Nellore cattle grazing tropical grass during the rainy season altered the profile of ruminal microorganisms.

The relative abundance of *F. succinogenes* and *S. ruminantium* was greater and that of *R. flavefaciens* was lower in animals fed additives compared with the control animals at day 28. Monensin is known to preferentially inhibit ruminal gram-positive bacteria (Weimer et al., 2008). This may help to explain the decrease in the relative abundance of *R. flavefaciens*, which are gram-positive bacteria, and therefore, are more sensitive to the inclusion of this feed additive in the diet. However, compared with day 28, feed additives inclusion decreased the relative abundance of *F. succinogenes* and *S. ruminantium* and increased in *R. flavefaciens* at day 118, which may be due to an adaptation of the microorganisms to the additives. Our findings are in line with those from Lee and Beauchemin (2014), who reported that some compounds, such as monensin, can effectively decrease CH_4 emission through modulation of ruminal microorganisms population in short term; however, it may be not effective in the long term due to a microbial adaptation do the feed additive. Additionally, Alexander et al. (2008) reported an increase in bacterial resistance in feedlot cattle receiving antimicrobials such as virginiamycin and monensin as growth promoters. Total Archaea relative abundance was not altered by the inclusion of feed additives in the diet. Similar results were reported by Schären et al. (2017), who did not observe a monensin effect on the archaea population of transition dairy cows.

Although it has been reported that, in ruminants, monensin can decrease CH_4 synthesis through the increase of propionate synthesis (Richardson et al., 1976; Callaway et al., 2003) caused by changes in the microbial population in the rumen, the alteration in the ruminal microorganisms observed in the current study was not followed by alterations on the molar proportion of propionate neither enteric CH_4 emission. Nevertheless, according to Arelovich et al. (2008), although monensin is usually associated with increases in ruminal propionate synthesis, factors such as feeding procedures, feed ingredients, and chemical composition of the diet can make animal response to dietary inclusion of monensin more variable. In the current study, the average enteric CH_4 emission is below of that established by the IPCC (2019) for growing steers in Latin America (112.7 vs. 129 g of CH_4/animal/day). Our findings are in line with those from Barbero et al. (2015), Neto et al. (2015), and San Vito et al. (2016), who reported 41 vs. 48, 46, and 43 kg of enteric CH_4/year from grazing cattle.

In the present study, blood glucose concentration was similar across treatments. It is well stablished that, when added to the diet of ruminants, monensin can increase ruminal synthesis of propionate and the supply of this glucogenic substrate to the hepatic tissue, causing an increase in glucose synthesis via gluconeogenesis in the liver (Ipharraguerre and Clark, 2003) and blood concentration of glucose. The absence of difference in blood glucose concentration may be due to the similar ruminal molar proportion of propionate among treatments. Similar results were reported by Harmon et al. (1993), who observed no effects of monensin supplementation on propionate and blood glucose concentration of steers receiving alfalfa hay. In addition, Vendramini et al. (2015), evaluating the effects monensin supplementation on beef cattle consuming ground stargrass (*Cynodon nlemfuensis*) hay, reported no differences in blood glucose concentration across treatments. Stephenson et al. (1997) suggested that in late-pregnancy cows, ionophores can alter glucogenic flux without affecting blood glucose concentration through the stimulation of insulin release. This may help to explain the similar blood glucose concentration and the increase in blood insulin concentration in animals fed monensin-containing diets.

There is scarce literature regarding carcass characteristics of supplemented cattle grazing tropical grass in the rainy season and receiving monensin and virginiamycin, most of the studies evaluate high-energy diets and feedlot animals. In the present study, no differences in initial and final BW and carcass characteristics were observed between control and feed additive-supplemented animals. Our findings are in line with those reported by Salinas-Chavira et al. (2009), who observed no effects of

virginiamycin and monensin supplementation on growth-performance characteristics of calf-fed Holstein steers. Similar results were reported by Lemos et al. (2016), who observed no differences in growth performance and carcass characteristics of finishing zebu cattle fed a no-roughage WSC-based diet supplemented with monensin and virginiamycin alone or combined. Additionally, Gibb et al. (2001), evaluating the effect of monensin and salinomycin on performance of cattle fed wheat- or barley-based diets, observed no difference in carcass characteristic across treatments. Although the present study observed no effects of treatments on carcass characteristics of the animals, the combination of additive supplementation decreased forage DMI without altering the ADG and total gain when compared with animals fed WA and VM, suggesting an improvement in the feed efficiency of the animals.

5. Conclusions

The use of monensin and virginiamycin combined alters the rumen microbial populations but does not decrease enteric CH_4 emission of the animals. However, it decreases forage dry matter intake without altering the average daily gain and total weight gain, leading to an increase in feed efficiency. Results from this study indicate an advantage in including feed additives in combination in the diet of supplemented Nellore cattle grazing tropical grass during the rainy season.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: E.E. Dallantonia and E. San Vito. Data curation: E.E. Dallantonia, Y.T. Granja-Salcedo and P.S. Castagnino. Formal analysis: E.E. Dallantonia, Y.T. Granja-Salcedo, A.G. Silva Sobrinho, R.A. Reis and T.T. Berchielli. Funding acquisition: T.T. Berchielli. Investigation: E.E. Dallantonia, J.D. Messana, E. San Vito, R.A. Reis and T.T. Berchielli. Methodology: E.E. Dallantonia, Y.T. Granja-Salcedo, J.D. Messana, P.S. Castagnino and A.G. Silva Sobrinho. Project administration: E.E. Dallantonia, Y.T. Granja-Salcedo and J.D. Messana. Resources: T.T. Berchielli. Supervision: R.A. Reis and T.T. Berchielli. Visualization: E.E. Dallantonia, L.G. Silva, J.D. Messana, L.F. Brito and A.R.C. Lima. Writing – original draft: E.E. Dallantonia, Y.T. Granja-Salcedo, J.D. Messana, L.F. Brito and A.R.C. Lima. Writing – review & editing: L.G. Silva, Y.T. Granja-Salcedo and J.D. Messana.

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