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RESEARCH ARTICLE

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Supplement levels and functional oils to replace virginiamycin for young bulls during early dry season on grasslands and finishing phase in feedlot systems

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Abstract

Aim of study: To assess the effects of replacing virginiamycin (VM) by functional oils (FO) from castor beans and cashew nut on beef cattle system during the early dry season (Experiment I) and during the finishing phase were evaluated the historical effect, keeping the treatments and methods intact (Experiment II).

Area of study: These experiments were conducted at the Forage Crops and Grasslands section of São Paulo State University, "Julio de Mesquita Filho" (Unesp–Jaboticabal, São Paulo, Brazil).

Material and methods: Two supplementation levels combined with two additives (four treatments in total) were evaluated: LSVM, low supplementation (0.3% body weight [BW]) with VM; LSFO, low supplementation (0.3% BW) with FO, HSVM, high supplementation (0.6% BW) with VM, and HSFO, high supplementation (0.6% BW) with FO. In both experiments, the experimental design was completely randomized with a 2 × 2 factorial arrangement (supplementation levels × additives).

Main results: In Exp. I, the additive effect of VM provided greater average daily gain (ADG, p=0.02), higher supplementation level resulted in higher ADG (p=0.04) and the greatest crude protein apparent digestibility (p=0.002). However, no effects were observed between supplementation levels, additives, and interactions (p≥0.11) on voluntary intake and ruminal parameters. In Exp. II, LSVM treatment resulted in lower dry matter intake (p=0.04). Animals maintained on LSFO during the early dry season exhibited lower carcass yield (p=0.004).

Research highlights: FO can be used to replace VM in beef cattle diet during the finishing phase in the feedlot without altering animal performance.

Additional key words: beef cattle; cardol; cardanol; cashew nut; castor beans; organic additive; ricinoleic acid.

Abbreviations used: ADF (acid detergent fiber); ADG (average daily gain); BW (body weight); CP (crude protein); DM (dry matter); DMI (dry matter intake); EE (ether extract); FCR (feed conversion ratio); FO (functional oils); HSFO (high supplementation with functional oils); HSVM (high supplementation with virginiamycin); iNDF (indigestible neutral detergent fiber); LSFO (low supplementation with functional oils); LSVM (low supplementation with virginiamycin); NDF (neutral detergent fiber); OM (organic matter); VM (virginiamycin).

Authors' contributions: Conceived, designed, and performed the experiments: DMR, AH, TLRA, LMD, RGL, JLR and RAR. Analysed the data: DMR, EPR, RPB and RAR. Statistical analysis: EPR and RPB. Contributed reagents/materials/analysis tools: JLR and RAR. Drafting of the manuscript: DMR, EPR and RAR. Wrote the paper: EPR, RPB and RAR. Supervising the work: RAR. Obtaining funding: JLR and RAR. All authors read and approved the final manuscript.

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Introduction

Many technologies have been used to improve beef cattle chain, which has resulted in system intensification. Among these, animal supplementation (Romanzini *et al.*, 2018) and feedlot use during the finishing phase can be highlighted. These technologies can reduce season effects, mainly in the dry season. Specifically, the use of the feedlot system to finish animals during the period with low forage availability would be more efficient if forage production potential was created in relation to heavier animals from the growing phase. This condition is of great importance for the viability of Brazilian beef cattle production systems (Barbero *et al.*, 2017).

Additives are important ingredients in beef cattle diets because of their beneficial action. These compounds can decrease the negative effects of diet changes caused mainly by the start of the finishing phase. During this phase, animals are fed with diets composed of high proportions of carbohydrates quickly fermentable, which could result in several changes in the rumen. These changes occur because of an increase in free glucose that elevates volatile fatty acid and lactic acid production, causing ruminal acidosis, laminitis, and tympanism (Millen *et al.*, 2009). Lemos *et al.* (2016) reported that virginiamycin additive improves animal performance, in addition to changing ruminal fermentation, resulting in decreased metabolic issues. According to Cocito (1979), virginiamycin is a non-ionophore antibiotic that acts on protein synthesis, specifically the 50S ribosomal subunit, thereby inhibiting the growth of gram-positive bacteria. However, some marketplaces, mainly in Europe, do not purchase animal products from countries that feed animals with these additives because of new rules regarding food security. These rules put forth by Council Regulation 2821/98 (EC, 1998) prohibit growth-promoting additives such as monensin and virginiamycin. Therefore, to meet the requirements of these countries, conventional additives need be replaced by plant extracts and essential functional oils (Fugita *et al.*, 2017).

This replacement is one way to overcome the problem of such marketplace bans; however, it may lead to undesirable consequences on animal performance, as their mechanisms of action on rumen fermentation are not yet fully defined. Therefore, new studies to understand their mechanisms of action are necessary. These plant essential oils could be used to replace traditional additives because they are secondary plant metabolites that normally have antimicrobial properties. These secondary metabolites are cardol and cardanol; and ricinoleic acid from cashew nut and castor beans respectively. According Nagabhushana *et al.* (1995), the compounds related to cardol and cardanol are phenolic compounds that act as monovalent ionophore. Lima *et al.* (2000) reported some antimicrobial effect from cardol, a compound from anacardic acid, in gram-positive bacteria as *Streptococcus mutans* and *Staphylococcus aureus*, and Amoratti *et al.* (2001) and Trevisan *et al.* (2006) reported both functions for cardanol compound, anti-inflammatory and antioxidant. In this way, considering the ricinoleic acid, that has a function similar to divalent ionophore (Vieira *et al.*, 2001), it has been studied to reduce digestibility, acetate:propionate ratio and methane synthesis, besides change the rumen bacteria resistance to ionophore. Consequently, because the functional essential oil composition, they could modulate ruminal fermentation and improve nutrient utilization by animals (Calsamiglia *et al.*, 2007). Therefore, the aim of this study was to investigate whether different inclusion levels of functional essential oils from cardol, cardanol and ricinoleic acid, to replace virginiamycin as additives, would change the metabolic parameters, animal performance and carcass yield of beef cattle.

Material and methods

Two experiments were performed: first, during the early dry season, when the tropical grasses started to decline in productivity and quality and second, during the finishing phase, using the feedlot system. Both experiments were conducted at the Forage Crops and Grasslands section of São Paulo State University, "Julio de Mesquita Filho" (Unesp–Jaboticabal, São Paulo, Brazil). This forage unit is located at 21°15′22″ S, 48°18′58″ W, at an altitude of 595 m a.s.l, and the climate is subtropical humid with dry winters and wet summers. The experimental procedures were approved by the Ethics, Bioethics, and Animal Welfare Committee of Unesp, Jaboticabal (protocol 022368/12).

Experiment I – Early dry season

Area, period, grazing method, and herbage characterization

Urochloa brizantha 'Marandu' (Marandu grass) was sown in the experimental area used during this phase. Eight experimental paddocks with feed bunks (60 cm linear per animal) and water drinkers were used. The evaluation period during this phase was from May to August 2015, with 14 d for adaptation to the diets and 90 d for evaluation. During this experimental phase, the pasture management adopted was continuous stocking, where at the beginning, the herbage mass available was close to 5 t of dry matter (DM).

All the herbage evaluations (herbage mass, morphological compounds, total DM, availability of leaf, and chemical composition) were performed according to Barbero *et al.*

(2015) and Delevatti *et al.* (2019). The mean values observed for herbage during the total experimental period were as follows: herbage mass, 4,539 kg DM/ha; leaf mass, 2,809 kg DM/ha; stem mass, 1,730 kg DM/ha; leaf:stem ratio, 1.89; and availability of leaf, 1.38 kg DM/kg body weight (BW). The values of herbage chemical composition obtained were: crude protein (CP), 15.9% DM; neutral detergent fiber (NDF), 64.7% DM; acid detergent fiber (ADF), 32.7% DM; indigestible neutral detergent fiber (iNDF), 24.8% DM; ether extract (EE), 1.49% DM; lignin, 4.45% DM; organic matter (OM), 89.6% DM; nitrogen insoluble in neutral detergent (NDICP), 34.7% CP; and nitrogen insoluble in acid detergent (ADICP), 10.0% CP.

Animals and treatments

Forty Nellore (Bos taurus indicus) bulls with a mean initial BW (IBW) of 403 ± 24 kg were used. Each treatment used two experimental paddocks, where 10 tester animals were allocated following a randomized distribution. The treatments studied were a combination of the additives virginiamycin (VM) and functional oils (FO) with low (LS) and high levels (HS) of supplementation, creating four treatments: (1) VM with 0.3% BW level of supplementation (LSVM) corresponding to 175 mg/kg of supplement; (2) FO with 0.3% BW level of supplementation (LSFO) corresponding to 2,260 mg/kg of supplement; (3) VM with 0.6% BW level of supplementation (HSVM) corresponding to 70 mg/kg of supplement; (4) FO with 0.6% BW level of supplementation (HSFO) corresponding to 810 mg/kg of supplement, with 98, 491, and 221 mg/kg of cardol, cardanol, and ricinoleic acid, respectively (Table 1).

Tester animals were weighed every 30 d after the adaptation period (14 d), always at 5:30 am, and after both a feed and water fast of 14 h. The weight was used to evaluate the gain during the period and to adjust the supplement supply. The total BW of animals in each paddock, during each experimental period, allowed the calculation of the stocking rate measured in animal units per hectare (AU/ha, 450 kg BW/ha).

Chemical analysis

All samples were dried at 55 ± 5 ºC to a constant weight and then ground through a 1 mm screen in a shear mill (Thomas-Wiley Laboratory Mill Model 4; H. Thomas Co., USA) for further processing. The analyses of DM

Table 1. Chemical composition and additive concentration of the supplement used to feed young bulls during early dry season (Experiment I)

Additive (mg/kg)[1]	Treatment[2]			
	LSVM	LSFO	HSVM	HSFO
Virginiamycin	175	-	70	-
Functional oils	-	2,260	-	810
Cardol	-	273	-	98.0
Cardanol	-	1,370	-	491
Ricinoleic acid	-	617	-	221
Chemical composition (%DM)				
CP	35.5	31.7	32.2	32.7
NDF	21.5	20.7	27.8	27.5
ADF	7.75	7.74	10.1	10.7
iNDF	12.24	11.4	15.0	14.8
EE	2.51	2.95	5.48	5.29
Lignin	3.22	2.10	2.96	3.03
OM	73.1	69.9	88.1	88.1
NDICP (%CP)	5.50	4.89	7.06	6.52
ADICP (%CP)	1.25	2.19	1.85	2.69

[1] Values in mg/kg DM of supplement. CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; iNDF: indigestible NDF; EE: ether extract; OM: organic matter; NDICP: neutral detergent-insoluble CP; ADICP: acid detergent-insoluble CP. [2]LSVM: low supplementation (3 g/kg BW) with virginiamycin; LSFO: low supplementation (3 g/kg BW) with functional oils; HSVM: high supplementation (6 g/kg BW) with virginiamycin; and HSFO: high supplementation (6 g/kg BW) with functional oils.

(method 934.01), OM (method 942.05) and EE (method 920.39) of all samples followed procedures described in AOAC (1990). Following the same reference, the level of CP was determined using the Dumas method of combustion, with a Leco F528 N analyzer (LECO Corporation, St. Joseph, MI, USA). The concentrations of NDF and ADF were determined following Mertens (2002), using an ANKOM2000 Fiber Analyzer (ANKOM Technologies, Macedon, NY, USA). The concentration of NDF from supplements was measured according to Van Soest *et al.* (1991) using alpha-amylase, as these feeds have high starch levels. Lignin was measured in the ADF residues after hydrolysis of the cellulose in 72% H_2SO_4 (Van Soest & Robertson, 1985). Forage residues of NDF and ADF were recovered and analyzed for CP to determine NDICP and ADICP. The concentrations of iNDF were determined as described by Casali *et al.* (2008). Samples were weighed and placed in the rumen of a cannulated steer for 240 h and subsequently analyzed for NDF as described earlier in this section.

Ruminal parameters

Four rumen-cannulated Nellore steers with a mean BW of 406 ± 39 kg were used. These animals were distributed in a 4 × 4 Latin Square experimental design (treatments × periods), where each animal stayed in paddocks joined to animals used in performance trials. The rumen-cannulated animals fed with different treatments were evaluated. The Latin Square design was performed twice: a second one was developed using the same treatments, animals, and period numbers. Four time periods, of 14 d each, were assigned, with the first 10 d used for the adaptation to diets and, both intake and nutrient digestibility evaluations were performed between days 8 and 14 of each period.

Herbage intake was estimated according to Delevatti *et al.* (2019), who considered fecal excretion and iNDF as internal markers. To estimate fecal excretion, 10 g of chromium oxide (Cr_2O_3) was used as an external marker. Cr_2O_3 was administered via the rumen cannula, after supplying supplement straight within the rumen at 11:00 h. This process occurred for 10 d, where the first 7 d were for adaptation and the last 3 d were to conduct fecal sampling. Fecal sampling was conducted twice daily at one of the following time combinations: 07:00 and 13:00 h; 09:00 and 15:00 h; and 11:00 and 17:00 h. After all samplings were completed, the samples were processed as described in section aforementioned for further analyses, maintaining individual identification of each sample. The estimation of iNDF in feces was conducted using samples from the grazing-simulation (hand-plucking) method from approximately 20 points/ha (Sollenberger *et al.*, 1995), following the method described by Casali *et al.* (2008). The total amount of daily supplement intake for each animal was measured by low and high levels of supplementation, with 0.3% BW and 0.6% BW, respectively.

To evaluate the pH and rumen ammonia nitrogen (RAN), the sampling of rumen fluid was performed during two consecutive days (d 12 and 13 of each experimental period) to reduce possible changes occurring during grazing cycles and in animal behavior each day. These samples were obtained at different times, 0, 2, 4, 6, 8, and 12 h after supplementation. The first sampling was performed before administration of the supplement, which occurred at 11:00 am. The ruminal fluid was collected manually from three different locations in the rumen. The material was filtered through three layers of cheesecloth, and the pH was measured using an electronic sensor (DM-23-DC model, DIGIMED, Digicrom Analytic, São Paulo, Brazil). The RAN was measured by the colorimetric method according to Chaney & Marbach (1962).

Volatile fatty acids (VFAs) were evaluated following the methods described by Delevatti *et al.* (2019), where the ruminal fluid was defrosted in the refrigerator overnight and centrifuged at 4 °C and 20,000×g for 30 min. The resultant supernatant was analyzed for VFA concentrations (Palmquist & Conrad, 1971) by gas chromatography (GC2014; Shimadzu Corporation, Kyoto, Japan) using an HP-INNO wax capillary column (30 m × 0.32 mm; Agilent Technologies, Loveland, CO, USA) at an initial temperature of 80 °C and a final temperature of 240 °C.

During the 14th d of each experimental period, urine was collected from cannulated steers by spot sampling before the supplementation time and 4 h later. The urine was filtered, and two aliquots were measured; the first with 10 mL was diluted in 40 mL of sulfuric acid (Valadares *et al.*, 1999), and the second with 40 mL was kept pure for further analyses of total nitrogen. Both the samples were stored at -20 °C until analysis.

Purine derivatives were determined using the first aliquot, by adding allantoin and uric acid (Delevatti *et al.*, 2019). Allantoin concentrations were measured according to Young & Conway (1942) and concentrations of uric acid and creatinine using diagnostic colorimetric test kits (Labtest Diagnostica SA, Lagoa Santa, MG, Brazil). All equations to obtain the final values were obtained from Delevatti *et al.* (2019). The second aliquot was analyzed using the Dumas method of combustion using a Leco F528 N analyzer (LECO Co.).

Blood collection was performed at the same time as that of urine sampling. Blood was collected directly from the caudal vein using a vacutainer with coagulation accelerator gel. The blood from each animal, at each time point (before supplementation and 4 h after supplementation), was centrifuged at 1,500 × g for 20 min to obtain serum and plasma. The first sample was stored at -20 ºC until analysis to determine the level of serum urea nitrogen. Following the methodology described by Valadares

et al. (1999), blood urea nitrogen was used to calculate nitrogen balance (NBal).

The daily urine volume was calculated according to the equation proposed by Chizzotti *et al.* (2006). The nitrogen balance, measured as g/day and % of intake, was calculated using equation (1):

$$NBal = NI - (NE_{fe} + NE_{ur}) \quad (1)$$

where NI is nitrogen intake, NE_{fe} is nitrogen excreted in feces, and NE_{ur} is nitrogen excreted in urine, and the latter two was measured in g/day.

Experiment II – Finishing phase

The total experimental period was 74 d, with 14 d for the adaptation to diets and 60 d for the evaluation conducted between October and December 2015. During this phase, all forty Nellore bulls, from the previous phase, were used. The animals were allocated into individual pens of 16 m² (4 × 4 m), with a roof, individual feed bunker, and water. The diets evaluated during this phase were formulated with a roughage/concentrate ratio of 30/70, to meet animal nutrition requirements for a daily weight gain close to 1.5 kg/day (Valadares Filho *et al.*, 2010) with 450 kg BW kept in the feedlot system. The roughage used was corn silage, and the concentrates were compounded by the same additives evaluated during the first phase, which had 29.2 mg/kg of VM + 31.7 mg/kg of monensin (VMMon) and 700 mg/kg of FO, compounded by 85, 424 and 191 mg/kg of cardol, cardanol and ricinoleic acid, respectively (Table 2).

The diet was supplied twice per day, at 7:00 and 14:00 h, during the same period as that in which the amount supplied to allow refusals between 5 and 7% of the total diet was adjusted. The adaptation period, first 14 d, was developed following restrictive dietary adaptation of 7 d, always maintaining the roughage/concentrate ratio of 30/70. On the first day, 1% BW of DM was supplied, and this value was increased daily by 0.2% BW of DM until animals had stabilized DM intake. On day 7 of adaptation, the animals reached a DM intake close to 2.2% BW, provided these animals were not allowed refusals of the total amount supplied on the last day. During the adaptation period between d 8 and 14, the diet amount supplied was controlled by refusal levels, ranging between 5% and 7%.

The treatments evaluated during the finishing phase were labelled the same as that in the previous phase to track the historic effect. Therefore, animals that received FO (LSFO and HSFO) during the dry season were also supplemented with FO during the finishing phase (FO concentration of 700 mg/kg DM). The animals that received VM in the dry season (LSVM and HSVM) had VMMon as the supplement in the finishing phase, with 29.2 and 31.7 mg/kg DM of VM and VMMon concentrations, respectively. The chemical composition of the diets was analyzed following the same methodologies described in the chemical analysis section previously mentioned.

The slaughter criterion was determined by the length of stay in the feedlot system (d 60), without accounting for the adaptation time for this phase. During the finishing phase, animal performance, such as average daily gain (ADG), DM intake (DMI), feed conversion ratio (FCR), and carcass yield from animals were evaluated. The ADG was obtained at the beginning (after the adaptation period) and at the end of the experimental period. The weighing was always conducted at 05:30 am, after a 14 h feed and water fast. The total BW gain was divided by the total number of days in the feedlot system to obtain ADG, measured as kg/day. The DMI was calculated by daily differences between the DM amount supplied and DM amount in refusals. The FCR was calculated considering the total DMI divided by BW gain during the total period. The carcass yield was calculated using the weight of the hot carcass after the slaughter process, and final BW was obtained from the last weighing (Barbero *et al.*, 2017).

Experimental design and statistical analysis

Experiment I – Early dry season

For animal performance, the experimental design was a completely randomized 2 × 2 factorial arrangement (additives [AD] × levels [L]). The paddocks were considered experimental units (n=8). Normality of errors and homoscedasticity of data were verified by PROC UNIVARIATE procedures, and significance was considered at a p-value<0.05 by Tukey's test. The interactions were evaluated between the levels and additives (AD × L). The model for these analyses was as follows:

$$Y_{ij} = \mu + a_i + b_j + (ab)_{ij} + \varepsilon_{ij}, \quad (2)$$

where Y_{ij} is the dependent variable, μ is the overall mean, ai is the additive effect, b_j is the level effect, (ab)ij is the interaction effect between additive and level, and ε_{ij} is the residual experimental error.

The analysis of the ruminal parameters was performed using a double Latin Square with repeated measurements as an experimental design, when the PROC MIXED procedure was developed. The treatments and time were considered fixed effects in the Latin Square, and animal and period were considered random effects. These Latin squares were performed twice at a time to consider statistical errors in the degrees of freedom. The data were tested for the best covariance structure considering the lowest Akaike Information Criterion scores and then included in the REPEATED statement to complete the analysis.

Table 2. Diet composition (chemical and centesimal composition) used during finishing phase of young bulls in feedlot system (Experiment II)

Additive (mg/kg)[1]	Corn silage	Concentrate[2]	
		VMMon	FO
Virginiamycin	-	29.2	-
Monensin	-	31.7	-
Functional oils	-	-	700
Cardol	-	-	85
Cardanol	-	-	424
Ricinoleic acid	-	-	191
Chemical composition (%DM)			
CP	9.57	15.1	15.8
NDF	46.6	29.8	30.0
ADF	29.5	17.3	18.1
iNDF	20.1	5.28	5.56
EE	3.41	2.70	2.86
Lignin	4.65	3.00	2.91
OM	95.7	94.8	94.3
NDICP (%CP)	16.6	13.3	13.8
ADICP (%CP)	6.97	6.31	4.84

[1] Values in mg/kg DM of supplement. CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; iNDF: indigestible NDF; EE: ether extract; OM: organic matter; NDICP: neutral detergent-insoluble CP; ADICP: acid detergent-insoluble CP. [2]VMMon: virginiamycin + monensin; FO: functional oils

Differences in means were determined by Tukey's test, when $p<0.05$. The statistical model was as follows:

$$Y_{ij(k)m}=\mu + SQ_m + PER_{im} + ANI(SQ_m)_j + \tau_{(k)} + \varepsilon_{ij(k)m}, \quad (3)$$

where $Y_{ij(k)m}$ is the dependent variable, μ is the overall mean, SQm is the Latin square effect, PER_{im} is the period effect, $ANI(SQ_m)j$ is animal j in the Latin square m effect, $\tau_{(k)}$ is the treatment k effect, $\varepsilon_{ij(k)}m$ is the residual experimental error, k is the treatment number that ranges from 1 to r, and m is the Latin square number that ranges from 1 to b.

Experiment II – Finishing phase

The experimental design was completely randomized, considering four treatments (animals historic from early dry season) and ten tester animal replicates, per treatment. The procedures were the same as those of Exp. I (animal performance analysis), without considering the interaction mentioned previously (AD × L). The model used in these analyses was as follows:

$$Y_{ij}=\mu + \tau_i + \varepsilon_{ij} \quad (4)$$

where Y_{ij} is the dependent variable, μ is the overall mean, τi is the treatment effect, and εij is the residual experimental error.

All analyses were performed using SAS® (v.XX; SAS Inst. Inc., Cary, NC, USA).

Results

Experiment I – Early dry season

The DMI of supplements was different ($p<0.001$) between the levels evaluated (LS and HS) (Table 3): between animals supplemented with higher levels (0.6% BW) of intake at 2.55 kg/day and those supplemented with 1.27 kg/day (0.3% BW). Considering herbage ($p=0.23$) and total DMI ($p=0.56$), there was no difference in both levels studied. Following these results, the nutrient intake (CP [$p=0.09$], NDF [$p=0.72$], and total digestible nutrient (TDN) [$p=0.18$]) was not altered in response to the treatments evaluated. However, although there was no difference (Table 3), CP and TDN intakes were 1.46 and 4.05 kg/day, respectively, for animals supplemented with 0.3% BW. Similarly, NDF at the lower intake level showed no differences ($p=0.64$) and was 4.73 kg/day for animals that

Table 3. Nutrient intake, apparent digestibility, and performance of young bulls fed on Marandu grass pastures with different supplement levels and additives during early dry season (Experiment I)

Variable	Level (L)		Additive (AD)		SEM	*p*-value		
	3 g/kg	6 g/kg	VM	FO		L	AD	L×AD
Animal intake (kg/day)								
DM supplement	1.27	2.55	1.91	1.91	0.12	**	1.00	1.00
DM herbage	7.10	6.19	6.84	6.46	0.38	0.23	0.61	0.88
DM total[1]	1.96	2.05	2.06	1.95	0.08	0.56	0.50	0.92
CP	1.46	1.75	1.65	1.55	0.09	0.09	0.55	0.99
NDF	4.94	4.75	4.97	4.73	0.25	0.72	0.64	0.83
TDN	4.05	4.69	4.46	4.28	0.24	0.18	0.70	0.99
Apparent digestibility (g/kg)								
DM	520.3	505.4	511.1	514.2	9.31	0.59	0.87	0.65
CP	714.9	753.0	731.6	737.7	8.93	0.002	0.82	0.57
NDF	491.7	435.6	460.8	464.9	10.01	0.01	0.89	0.75
OM	559.3	564.8	561.3	563.0	8.40	0.49	0.81	0.56
Animal performance								
Stocking rate[2]	4.17	5.18	4.72	4.64	0.18	0.02	0.85	0.94
ADG[3]	0.61	0.77	0.78	0.60	0.06	0.04	0.02	0.71

VM: virginiamycin; FO: functional oils; SEM: standard error of mean. DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; TDN: total digestible nutrient; OM: organic matter. ** $p<0.0001$. [1]DM total: percentage of BW. [2]Stocking rate: animal units (AU=450 kg BW/ha). [3]ADG: average daily gain (kg/day).

were fed FO (Table 3). Differences in apparent nutrient digestibility could be seen for CP (p=0.002) and NDF (p=0.01) between the levels evaluated. However, the DM (p=0.59) and OM (p=0.49) digestibility did not differ for all evaluations (additive, levels, and interactions).

For animal performance, the ADG was different for both levels (p=0.04) and additives (p=0.02) but did not result in a significant interaction effect (p=0.71). Higher values of ADG were observed for high supplementation levels (0.6% BW – 0.77 kg/day) and for the conventional additive (VM – 0.78 kg/day). Furthermore, the higher supplementation level (0.6% BW) resulted in higher stocking rate, 5.18 AU/ha (p=0.02, Table 3); however, no differences were observed between additives (p=0.85).

Concerning ruminal parameters (Table 4), differences were observed only between time samples for pH ($p<0.001$). The evaluations between the levels and additives did not result in differences in both pH (p=0.24 and

Table 4. Ruminal parameters of young bulls fed on Marandu grass pasture with different supplement levels and two additives (virginiamycin and functional oils) during early dry season (Experiment I)

Variable	Level (L)		Additive (AD)		SEM	*p*-value[1]					
	3 g/kg	6 g/kg	VM	FO		L	AD	Time (T)	L×AD	L×T	AD×T
pH	6.43	6.38	6.42	6.40	0.03	0.24	0.57	<0.0001*	0.35	0.05	0.98
RAN[2] (mg/dL)	30.96	33.13	31.59	32.49	1.16	0.36	0.69	0.15	0.62	0.40	0.92
Volatile fatty acids (mM/L)											
Acetic acid	83.51	84.74	82.38	85.57	1.51	0.68	0.26	0.77	0.49	0.36	0.75
Propionic acid	20.86	20.00	19.83	21.01	0.44	0.34	0.19	0.58	0.94	0.16	0.65
Butiric acid	10.48	10.33	10.25	10.55	0.22	0.74	0.53	0.43	0.54	0.21	0.85
Isobutiric acid	1.07	1.05	1.03	1.09	0.02	0.69	0.16	0.16	0.57	0.37	0.30
Isovaleric acid	1.77	1.76	1.73	1.81	0.04	0.91	0.38	0.30	0.85	0.76	0.57
Valeric acid	1.21	1.13	1.13	1.21	0.04	0.29	0.32	0.23	0.52	0.07	0.45
Ac:Prop rate[3]	4.13	4.32	4.28	4.18	0.05	0.06	0.36	0.69	0.38	0.15	0.29

VM: virginiamycin; FO: functional oils. SEM: standard error of the mean. [1]Time effect after sample harvest (T): 0, 2, 4, 6, 8, and 12 h. L×AD: interaction between level and additive; L×T: interaction between level and time; AD×T: interaction between additive and time. * Cubic effect. [2]RAN: rumen ammonia nitrogen. [3]Ac:Prop rate: acetic acid:propionic acid rate.

p=0.57) and RAN (*p*=0.36 and *p*=0.69). The means obtained for pH and RAN between treatments were 6.41 and 32.04 mg/dL, respectively. There was no difference in VFA levels. However, a tendency could be reported in the acetic acid:propionic acid rate in response to level effect (*p*=0.06); this occurred for animals supplemented with a higher supplementation level (0.6% BW), which had a greater acetic acid:propionic acid ratio of 4.32, against the ratio of 4.13 from animals supplemented with 0.3% BW (Table 4).

There was no difference between all evaluations for neither EMICS (*p*=0.66) nor NBal (*p*=0.11) (Table 5). The mean of EMICS, measured in g microbial nitrogen/kg TDN, was 16.36, and when measured as microbial CP/kg TDN, the mean was 102.24. The means of NBal were around 161.60 g/day and 60.75% of intake (Table 5). In this way, no interaction effect was observed between the supplementation level and additive for all variables analyzed.

Experiment II – Finishing phase

Differences were not observed in the IBW (*p*=0.19) or final BW (FBW; p=0.64) from animals finished in the feedlot system (Table 6). The animals started this phase with a mean weight of 487 kg and finished with 583 kg. The DMI was different between the treatments evaluated for both units, kg/day (*p*=0.04) and %BW (*p*=0.002). When DMI measured in kg/day was evaluated, animals from the FO treatments (LSFO and HSFO) had greater values than those of animals from the LSVM treatment; however, the DMI of animals from the HSVM treatment did not differ from that of the first group of animals. The difference between treatments is emphasized when DMI was expressed in % BW: animals that were fed FO (LSFO and HSFO) had a DMI that was 12.6% more than that of animals that were fed VM (LSVM and HSVM).

The ADG and FCR were not different (*p*=0.20 and *p*=0.17, respectively) between the treatments evaluated. The means obtained for these variables were 1.601 kg/day and 7.28 for ADG and FCR, respectively (Table 6). No difference was observed (*p*=0.22) in the carcass weight obtained from animals finished in the feedlot system, and the mean weight was 334 kg. However, the carcass from the animals fed during the early dry season with LSFO resulted in a lower yield (55.8%) when compared with carcass from other treatments (the average of LSVM, HSVM, and HSFO was 57.7%, Table 6).

Discussion

Experiment I – Early dry season

In grazing beef cattle systems, herbage DMI can change because of certain factors such as grazing intensity (Barbero *et al.*, 2015) and energy supplementation (Moore *et al.*, 1999). In this study, during the early dry season (Exp. I), evaluation of different supplementation levels resulted in contrasting DMI of supplements calculated for the animals. However, a crucial point to be highlighted is that if animals provided with a low supplementation level (0.3% BW) had the same total DMI, it could be understood that an increase in herbage DMI caused these results, which is consistent with the results of previous studies. We found differences in behaviour patterns of animals in each treatment. Casagrande *et al.* (2011) reported that animals have a constant grazing behaviour after they are adapted to management. The use of FO did not result in changes in animal behaviour, as was demonstrated by Ornaghi *et al.* (2017); the adjustments in animal intake to allow similar results between treatments actually occurred because of changes in herbage DMI.

Although this experiment was conducted during the early dry season, the forage quality was high, considering historic tropical grass data, which reported CP 15.9%, NDF 64.7%, ADF 32.7%, iNDF 24.8%, and lignin 4.45% of DM (Poppi & Mclennan, 2007). According to this study, the higher the pasture quality, the lower the difference in response obtained by supplementation of different kinds or levels. Thus, knowing that animals kept in grazing systems should obtain the total or most of their nutrients

Table 5. Efficiency of microbial synthesis and nitrogen balance of young bulls fed on Marandu grass pastures with different supplement levels and two additives (virginiamycin and functional oils) during early dry season (Experiment I)

Variable[1]	Level (L)		Additive (AD)		SEM	*p*-value		
	3 g/kg	6 g/kg	VM	FO		L	AD	L×AD
EMICS1	17.04	15.68	14.94	17.78	1.52	0.66	0.37	0.78
EMICS2	106.48	98.00	93.37	111.11	9.52	0.66	0.37	0.78
NBal (g/day)	140.06	183.03	167.36	156.23	13.07	0.11	0.67	0.95
NBal (% of intake)	57.72	63.77	60.54	60.95	2.09	0.16	0.92	0.82

VM: virginiamycin; FO: functional oils; SEM: standard error of the mean. [1]EMICS1: g microbial nitrogen/kg total digestible nutrient (TDN); EMICS2: g microbial crude protein/kg TDN; Nbal: nitrogen balance.

Table 6. Initial body weight (IBW), final BW (FBW), dry matter intake (DMI), yield and carcass weight, average daily gain (ADG) and feed conversion ratio (FCR) of Nellore bulls finished in feedlot system following consumption of historic feed during early dry season (Experiment II)

Variable[1]	Treatment				*p*-value
	LSVM	LSFO	HSVM	HSFO	
IBW (kg)	480 ± 21	474 ± 26	506 ± 20	488 ± 31	0.19
FBW (kg)	574 ± 24	582 ± 34	595 ± 21	582 ± 36	0.64
DMI (kg/day)	10.36 ± 1.20b	12.05 ± 1.17a	11.14 ± 1.01ab	11.80 ± 0.80a	0.04
DMI (%BW)	1.96 ± 0.16b	2.27 ± 0.13a	2.02 ± 0.15b	2.20 ± 0.13a	0.002
Carcass yield (%)	57.11 ± 0.59a	55.83 ± 0.94b	58.10 ± 0.76a	58.00 ± 1.90a	0.004
Carcass weight (kg)	327.0 ± 13.61	325.0 ± 23.53	346.0 ± 10.16	337.0 ± 25.38	0.22
ADG (kg/day)	1.564 ± 0.16	1.788 ± 0.18	1.486 ± 0.18	1.566 ± 0.30	0.20
FCR	6.70 ± 0.83	6.95 ± 1.07	7.61 ± 0.83	7.86 ± 1.42	0.17

LSVM: supplementation with 3 g/kg BW with virginiamycin (VM) in the early dry season and VM + monensin at the finishing phase; LSFO: supplementation with 3 g/kg BW with functional oils (FO) in the early dry season and FO at the finishing phase; HSVM: supplementation with 6 g/kg BW with VM in the early dry season and VM + monensin at finishing phase; HSFO: supplementation with 6 g/kg BW with FO during the early dry season and FO at the finishing phase. [1]IBW: initial BW; FBW: final BW; DMI: DM intake; ADG: average daily gain; FCR: feed conversion ratio. [ab] Means with different superscript letters are different by Tukey's test ($p<0.05$).

from herbage (Barbero *et al.*, 2015), the nutrient intake (CP, NDF, and TDN) measured from animals in this study would have to be similar to herbage DMI. The increase in supplement intake could be explained by the greater CP availability, and also, due to the nitrogen fractions more quickly metabolized (Orskov *et al.*, 1980), could cause an improvement in the apparent digestibility of CP. Milis & Liamadis (2007) reported that effects of CP levels on digestibility of nutrients are variable and depend on factors such as levels and sources of protein. Thus, animals that were fed a higher level of supplementation had greater and quicker protein digestibility, which could cause an increase in apparent CP digestibility. Regarding the apparent digestibility of NDF, Valadares Filho *et al.* (2016) reported that an improvement in the concentrate levels supplied to animals, expressed by the interaction with voluntary DMI, has negative effects on fiber digestibility. As the animals supplemented with 0.6% BW had greater DM supplement intake, the responses from our study are consistent with previous findings. Specifically, in terms of FO, there is limited information about the effect of the addition of these compounds on ruminant diets. Ornaghi *et al.* (2017) noted an absence of differences among diets, such as in *in vitro* digestibility, with the use of these natural additives, which is consistent with our results.

The high ADG obtained using high supplementation levels can be understood from previous studies, but the differences in ADG from the additives used could be explained by the specific compounds in each additive. FO is a secondary metabolite produced by plants, which can affect animal performance at different points, from modulation of microbiota to the disturbance in the colonization of substrates (Calsamiglia *et al.*, 2007). Castor oil acid and cashew nutshell liquid, both used as ingredients to compose the functional essential oils additive evaluated in this study, have ricinoleic acid, anacardic acid, cardanol, and cardol as the main components (Zotti *et al.*, 2017). These compounds have antimicrobial activity, especially the fatty acids in castor oil, which could be described as inhibitors of biohydrogenation and methane production and have some effects on gram-positive bacteria (Morales *et al.*, 2012). Thus, all activities are related to the improvement of animal performance; however, the low supplementation levels during this phase (0.3% and 0.6% BW) and the limitations of FO, such as low water solubility and stability (Rai *et al.*, 2017), could have caused the lower ADG compared to VM. Specifically, its use as a feed additive to mainly reduce acidosis risk makes it highly prevalent in Brazilian feedlot systems (Millen *et al.*, 2009). This compound is also used for other purposes, as a growth promoter and for disease prevention (Benatti *et al.*, 2017). Some studies evaluating VM reported high ADG with its use; Costa *et al.* (2018) reported an improvement of 14% using VM as an energy supplement. In this study, the improvement was 30% among the different additives.

Ruminal parameters have an important effect on animal performance; however, the results from this study did not show differences in these variables, except in pH between different sampling times. The minimum pH value to cause a reduction in cell wall degradation is 6.2 (Hoover, 1986). Changes occurring during these sampling times were expected and were caused by the ingestion behavior and timing of grazing of the steers. Grazing activity typically increases at the end of the afternoon (Casagrande *et al.*, 2011). This could possibly contribute to pH reduction, which intensifies 6 h after ingestion (Owens & Goetsch, 1993). Another important parameter that can alter microbial synthesis is RAN; some studies have

reported the minimum values needed to maintain and improve ruminal digestion in tropical pastures. Detmann *et al.* (2014) reported 6.3 mL/dL for tropical conditions similar to that obtained in this study, and it should be noted that all the means in this study were above this value. A possible association could be the similarity in another variable, such as NBal (Broderick *et al.*, 2010).

The similarity of pH values between the analyzed effects (levels and additives) can be related to the absence of differences in VFA. Total VFA concentration is directly associated with dietary composition and intake level; both characteristics are similar if herbage is considered as the main feed source, regardless of supplementation levels. Similarly, Cardozo *et al.* (2006) also reported that extracts composed of other types of FO had no effect on total VFA concentration, which is consistent with our results. Regarding EMICS and NBal, no difference was observed, despite the known antimicrobial effect of FO, probably because of the levels supplied to the animals during this phase. This condition was reported by other studies (Molero *et al.* 2004; Newbold *et al.*, 2004), where the blend of essential oils when used resulted in small and variable changes, depending of the feed being degraded, type of ration fed to the animals, and length of the adaptation period. In this way, Benchaar *et al.* (2006) affirmed that when dairy cattle were fed with a blend of essential oil (compounded by thymol, eugenol, vanillin, and limonene), resulted an increase on ruminal pH and ADF digestion, however no changes were observed to VFA, NBal, protozoa counts and animal performance. Therefore, according Calsamiglia *et al.* (2007), studies *in vivo* similar to this should be performed to determine between other points, the e the optimal dose in units of the active component and the effects on animal performance.

Experiment II – Finishing phase

The initial BW of the animals showed that all treatments had similar effects during the growing phase (Exp. I), allowing the animals to begin the finishing phase under the same conditions. Traditionally, during the finishing phase, ruminants are fed with diets containing high amounts of fermentable carbohydrates in feedlot systems. This situation can alter ruminal conditions, decrease rumen pH values drastically, and cause ruminal acidosis due to the accumulation of acids in the rumen (González *et al.*, 2012).

The adequate adaptation period and maintenance of the supplementation history of animals allowed the observation of differences in DMI between animals from each treatment. The higher DMI measured in animals that were fed FO can be attributed to the palatability properties of this compound, and also be associated to the slight improvement on rumen fermentation. Franz *et al.* (2010) reported the presence of volatile and odorant compounds in FO, which could determine its potential use as a feed additive in animal nutrition.

During the finishing phase, when high-grain diets are used, natural additives have the potential to modulate rumen fermentation (Valero *et al.*, 2014). Therefore, these additives, represented by FO in this study, can be used as natural products to replace ionophores and growth promoters (monensin and VM, respectively) in beef cattle production without future problems in trade to specific marketplaces with rigorous food safety bans (Ornaghi *et al.*, 2017).

The differences in carcass yield could be due to the historic feed supplied during the growing period, when the young bulls were fed with lower supplementation levels combined with FO as the additive, resulting in a lower ADG, whereas the other treatments promoted higher ADG. The animals from this treatment probably had high deposition of lean tissue and consequently improved carcass yield. This is supported by the ADG during the finishing phase, which occasionally remained unchanged during the feedlot. Regarding these results, our study did not agree with the findings from other studies that reported an improvement in animal performance (Ornaghi *et al.*, 2017) and feed efficiency (Valero *et al.*, 2014; Fugita *et al.*, 2017); however, these differences can be related to the compositions of each additive used in these studies. Besides decreasing methane production, which improves energy use (Ornaghi *et al.*, 2017), FO from different plants have different organic actions, as they have the potential to modulate rumen fermentation according to antimicrobial activity of each FO used (Geraci *et al.*, 2012). These actions can cause different responses in ruminants. Thus, more research on the effects of FO from plant extracts needs to be developed to refine their use in animal diets.

Hence, the supplementation level of 0.6% BW and VM as an additive increased beef cattle performance in grasslands during the early dry season when compared to FO, a mix of cardol, cardanol, and ricinoleic acid. Young bulls receiving 0.3% BW of supplement this mix of FO during this phase on grasslands exhibited lower carcass yield after the finishing phase in the feedlot. The mix of cardol, cardanol, and ricinoleic acid (FO) can replace VM as an additive for beef cattle during the finishing phase in the feedlot, without changing the ADG and FCR.

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