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Article

Responses of *Bos indicus* cattle to phytogenic feed additives compared with monensin in feedlot diets

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Abstract:

Two experiments assessed the responses of *Bos indicus* cattle fed monensin (MON), *Stryphnodendron adstringens* extract (BBT), a blend of essential oils (BEO), and a blend of functional oils (BFO). Feed additives were added to a total mixed ration (TMR) with a 20:80 roughage to concentrate ratio. Experiment 1, lasting 104 d, used a randomized complete block design. One hundred fourteen (114) crossbred bulls (336 ± 26 kg BW) were assigned to four treatments: MON (30 mg/kg DM), BBT (1500 mg/kg DM), BEO (118 mg/kg DM), and BFO (250 mg/kg DM). MON resulted in the lowest dry matter intake. Average daily gain, final body weight, and carcass traits were similar across treatments. Feed efficiency was 13 %, 11 %, and 4 % greater with MON compared to BBT, BEO, and BFO, respectively. Experiment 2 employed a 5×5 Latin square with five ruminally fistulated *Bos indicus* steers to test the same four treatments and a control diet without additives. Each period lasted 14 d. Apparent nutrient digestibility and concentrations of volatile fatty acids and NH_3-N were similar among treatments. BBT and BFO had the lowest (6.54) and highest (6.75) ruminal pH values, respectively. Monensin showed superior feed efficiency despite similar nutrient digestibility and rumen fermentation profiles compared to phytogenic additives.

Keywords*:* Growth performance, Carcass characteristics, Nutrient digestibility, Plant extracts, Rumen fermentation.

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Introduction

The main challenge in the livestock industry has been to sustain the high efficiency in animal performance, which is critical for profitability, while using sustainable practices that suppress risks to human health and the environment. Feed efficiency in ruminants is improved by reducing losses of the energy consumed. It can be achieved, among other factors (e.g., genetic and environmental improvement), by feeding diets that include compounds able to alter ruminal fermentation positively (e.g., ionophores such as monensin).

Phytogenic compounds (e.g., tannins, essential oils, and functional oils) have specific antimicrobial activities that modulate rumen fermentation and may improve nutrient utilization[1,2]. Therefore, replacing compounds (including antibiotics) currently used in

ruminant nutrition with naturally-occurring phytochemicals can enable sustainable livestock production advances worldwide. However, large-scale and robust *in vivo* studies are still needed to provide scientific support for the development of novel and to improve commercially available phytogenic products with productive and health benefits to ruminants.

Tannins are plant polyphenols that can bind with and precipitate proteins[3]. The bark extract from barbatimão (*Stryphnodendron adstringens*), a tree native to the Brazilian Cerrado, has a high concentration of condensed tannins and showed marked antimicrobial activity when the ruminal fermentation of high and low roughage diets was evaluated *in vitro*[4]. Essential oils are mixtures of volatile metabolites from plants obtained through steam distillation[1], which have several targets in the microbial cell and different modes of action, thus preventing resistance development. The diversity and synergistic interactions of plant secondary metabolites, therefore, present an advantage compared with using individual compounds that has a single mode of action[5]. Functional oils are fatty acids with properties (e.g., selective antimicrobial activity) that exceed their nutritional (i.e., energetic) value[6].

Therefore, it was hypothesized that feeding a condensed tannin-rich plant extract such as barbartimão (BBT, *S. adstringens*), a blend of essential oils (BEO), or a blend of functional oils (BFO) to finishing cattle may enhance rumen fermentation through selective antimicrobial activity, thus increasing the energy supply with positive effects in feed efficiency. Accordingly, the present study aimed to assess the growth performance, carcass characteristics, nutrients digestibility, and rumen fermentation responses of finishing cattle fed BBT, BEO, and BFO compared with monensin (MON).

Material and methods

Experimental procedures were reviewed and approved by the Ethics Committee on Use of Animals of the Universidade Federal de Goiás (UFG), approval number 89/14.

Experiment 1

Animals and experimental design

One hundred fourteen (114) *Bos indicus* crossbred bulls (predominantly Nellore; 336 ± 26 kg average initial body weight [BW]; average 22-mo old) were assigned to 20 pens (soil-surface pens; 7.7 m deep × 9.7 m wide; 9.7 m of linear bunk space) in a randomized complete block design. Bulls were ranked by ascending BW and assigned to BW blocks (average initial BW: block 1= 303 ± 9.4 kg; block 2= 319 ± 2.7 kg; block 3= 332 ± 6.6 kg; block 4= 350 ± 4.9 kg; block 5= 375 ± 14.1 kg). Bulls within a block were assigned randomly to pens (5 or 6 bulls/pen), and pens within a block were assigned randomly to 1 of 4 dietary treatments. Pen, containing 5 or 6 bulls each, was considered the experimental unit for all variables studied (n= 5 pens per treatment). The total of bulls assigned to each treatment was 29 for MON and BBT, and 28 for BEO and BFO.

Bulls were removed from feed (but allowed water) overnight (approximately 14 h), identified with numbered ear tags, individually weighed, vaccinated for clostridial diseases (Poli-Star; Vallée SA, São Paulo, SP, Brazil), and injected with a supplemental source of vitamins (ADE Injectable; Pfizer, São Paulo, SP, Brazil). Cattle also received albendazole sulfoxide (Agebendazol; Agener União, Embu-Guaçu, SP, Brazil), and ivermectin (Absolut; Vallée S.A., São Paulo, SP, Brazil), both for deworming purposes.

Treatments

The experimental treatments consisted of the use of four different additives, with monensin being the only one not of natural origin, and therefore considered the control. The treatments evaluated were MON: 30 mg/ kg of monensin (Rumensin-200; Elanco Animal Health, São Paulo, SP, Brazil); BBT: 1.500 mg/kg of bark extract from barbatimão (*Stryphnodendron adstringens*); BEO: 118 mg/kg of the microencapsulated blend of essential oils (carvacrol, cinnamaldehyde, and eugenol) and pepper extract (capsaicin from capsicum oleoresin); BFO): 250 mg/kg of the microencapsulated blend of functional oils (castor oil, copaíba oleoresin, and cashew nut shell liquid). Doses of MON, BEO, and BFO were recommended by the manufactures.

The crude extract from barbatimão (*S. adstringens*) was provided by the Natural Products Research Laboratory (LPPN) of the Faculdade de Farmácia-UFG (Goiânia, GO, Brazil). Barks from barbatimão trees were dried in a forced-air oven at 40 ± 5 °C for 48 h, ground using a Wiley mill (R-TE-625; Tecnal, Piracicaba, SP, Brazil) to pass through a 1-mm screen, macerated for 24 h with a hydroethanolic solution (80:20, ethanol to water ratio) and percolated at room temperature. This liquid extract was dried using a forced-air oven at 40 ± 5°C for 48 h. The barbatimão extract contained 440 g/kg of total tannins (analyzed as described by Sousa *et al*[7]. Treatments (feed additives) were mixed previously with soybean meal and then mixed with other dry ingredients (soybean meal, soybean hulls, ground corn, urea, and mineral supplement) to make a concentrate feed, as described in the next section.

Feeding management

Bulls were fed once daily (0800 h) and allowed *ad libitum* access to feed and water for 104 d. The finishing diet was formulated using the NASEM[7]. Bulls were gradually adapted to the finishing diet over a 14-d adaptation phase. The inclusion of roughage (sugarcane bagasse) in the total mixed ration (TMR) was reduced as follow: from d 1-7, bulls were fed a diet containing roughage to concentrate ratio of 30:70 (dry matter [DM] basis); d 8-14, cattle were fed a 25:75 diet; d 15 onward, cattle were fed the finishing diet (20:80; Table 1). All bulls received their respective treatments (contained in the concentrate feed) since d 1. Thus, data from the adaptation phase (i.e., d 1 to 14) were included in the statical analyses of DM intake and growth performance responses.

Table 1: Ingredients and nutrient composition of experimental diet

Item	Content
Ingredients, g/kg of DM	
Sugarcane bagasse	194.9
Ground corn	511.5
Soybean hulls	236.3
Soybean meal	12.2
Urea	14.6
Mineral supplement[1]	18.3
Premix with additives[2]	12.2
Nutrient composition	
Dry matter[3], g/kg of as fed	810 ± 9.9
Ether extract[3], g/kg of DM	26 ± 2.3
Crude protein[3], g/kg of DM	162 ± 6.3
NDF[3], g/kg of DM	298 ± 11.7
ADF[3], g/kg of DM	127 ± 10.3
Ash[3], g/kg of DM	33 ± 0.9
RDP[4], g/kg of DM	83.6
NEm[4], Mcal/kg	1.64
NEg[4], Mcal/kg	1.04

[1] Contained 130 g/kg Ca, 60 g/kg P, 111 g/kg Na, 6 g/kg Mg, 20 g/kg S, 80 mg/kg Co, 1,000 mg/kg Cu, 600 mg/kg Fe, 80 mg/kg I, 600 mg/kg Mn, 8 mg/kg Se, and 4,000 mg/kg Zn.

[2] Premixture of soybean meal with feed additives: MON= 30 mg/kg of monensin; BBT= 1.500 mg/kg of extract from barbatimão (*Stryphnodendron adstringens*); BEO= 118 mg/kg of the blend of essential oils (carvacrol, cinnamaldehyde, and eugenol) and pepper extract (capsaicin from capsicum oleoresin); BFO= 250 mg/kg of the blend of functional oils (castor oil, copaíba oleoresin, and cashew nut shell liquid).

[3] Analyzed composition, average ± standard deviation.

[4] Calculated based on nutrient analysis of each dietary ingredient using NASEM[(7)] equations (RDP = rumen degradable protein, NEm= net energy for maintenance, and NEg= net energy for gain).

The amount of concentrate feed needed in experiments 1 and 2 was manufactured in a commercial feed mill (Ganho Nutrição Animal; Goiânia, GO, Brazil) using the same lot of ingredients (soybean meal, soybean hulls, ground corn, urea, and mineral supplement) and the premixtures containing treatments. The concentrate feed was packed into 40-kg-capacity polypropylene bags labeled by treatment and stored at the research facility. At the feeding time, the concentrate feed was mixed with sugarcane bagasse using a 3 m^3-capacity horizontal tractor-pulled mixer (Siltomac 203; São Carlos, SP, Brazil). Dietary DM content was adjusted weekly to 650 g/kg with water addition to enhance intake and avoid feed sorting behavior. Bunks were visually evaluated at 0700 h daily to estimate the number of orts in each pen. Bunks were managed such that orts do not exceed 5 % of daily intake.

Sample collection, laboratory analyses, and measurements

Throughout the experiment, feed offered and orts were sampled weekly from each of the 5 pens/treatment, composited by treatment, and stored at −20 °C. A subsample of each weekly dietary treatment composite was obtained for DM analysis by drying in a forced-air oven for 24 h at 100 ± 5 °C; the DM value was used to calculate daily DMI by subtracting orts from feed offered (on DM basis). Ingredients were also sampled weekly and stored at −20 °C. At the end of the study, samples were thawed, composited by 28-d, dried in a forced-air oven at 55 ± 5 °C for 72 h, ground using a Wiley mill (Tecnal TE-650; Tecnal Equipamentos Científicos, Piracicaba, SP, Brazil) to pass through a 1-mm screen, and analyzed in duplicate according to AOAC[8] methods for laboratory DM (method 930.15), ash (method 942.05), crude protein (nitrogen × 6.25; N method 990.03), and ether extract (EE; method 945.16). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined in composited samples sequentially [9] using a heat-stable α-amylase (A3306; Sigma Chemical. Co., St. Louis, MO, USA), sodium sulfite omitted, and expressed exclusive of residual ash.

Aiming to assess ADG and feed efficiency (i.e., ADG/DMI), bulls were individually weighed one day before the beginning (d 1) and at the end (d 104) of the experiment, after overnight withdrawal of feed (approximately 14 h) but allowed access to water. At the end of the experiment, bulls were slaughtered in a commercial packing plant (JBS; Goiânia, GO, Brazil; 12 km from the research facility). Carcasses were individually identified, separated into two symmetrical sections, and weighed to obtain the hot carcass weight (HCW). Dressing percent (DP) was calculated by dividing the HCW by the final BW. After chilling for 24 h at 4 °C, left-side carcasses were ribbed between the 12th and 13th ribs, and the 12th-rib fat thickness was measured using a digital caliper (0 to 150 mm, accuracy 0.01 mm; Cingda HT0403-A1; Cingda Industry Co, Nanjing, JS, China).

Statistical analyses

Data were analyzed using the "easyanova" package[10] of R software[11] as a randomized complete block design with 4 treatments (feed additives) and 5 blocks (initial BW). Pen (n= 20) was the experimental unit for all variables assessed. The DMI recorded daily during the adaptation phase was analyzed as a repeated measure throughout days on feed. A compound symmetry covariance structure was used for the repeated measure analysis. This covariance structure showed the lowest Akaike information criterion value. Statistical differences were declared at $P<0.05$, and trends were discussed at $P\leq0.10$. The following model was used: $Y = \mu + Bi + Tj + eij$, in which μ = overall mean, Bi = block effect (i = 1 to 5), Tj = treatment effect (j = 1 to 4), and eij = residual error. The Tukey comparison test was used to test for

differences between means ($P<0.05$). Means are presented as least squares means with the standard error of the mean (SEM).

Experiment 2

Animals, experimental design, and treatments

Five (5) ruminally fistulated *Bos indicus* crossbred steers (predominantly Nellore; 275 ± 15.5 kg average BW at the beginning of the experiment) were housed in individual pens in a 5 × 5 Latin square design. Experimental periods were 14 d long and consisted of 10 d of adaptation to treatments and 4 d of measurements. Steers were submitted to the same feeding management applied to bulls in Exp. 1. Treatments from Exp. 1 plus one additional control treatment with no additives (CTL) were evaluated in the metabolism experiment.

Apparent total-tract nutrient digestibility

Feed and orts were recorded and sampled daily for individual steers from d 11 to 14. Fecal samples (approximately 200 g) were collected from the rectum of each steer twice daily (early morning and late afternoon) from d 11 to 13. All samples were stored at −20 °C. After the experiment, samples were thawed, composited for each steer by period, dried in a forced-air oven at 55 ± 5 °C for 72 h, and ground using a Wiley mill (R-TE-650; Tecnal Equipamentos Científicos, Piracicaba, SP, Brazil) to pass through a 1-mm screen. The chemical composition of feed, orts, and feces was analyzed as described in Exp. 1. Aiming to estimate daily fecal output (kg of DM), each steer received 5 g/d of chromium oxide (Cr_2O_3 [12]) via rumen fistula from d 1 to 13 of each experimental period. Apparent total tract digestibility of nutrients was calculated using the following equation: 100 − 100 × [(concentration of Cr_2O_3 fed/concentration of Cr_2O_3 in feces) × (concentration of nutrient in feces/concentration of nutrient in feed)].

Rumen fermentation

Rumen fluid was collected from the cranial, ventral, and caudal areas of the rumen, pooled into a 2-L-capacity container, and filtered through a cotton fabric. Samples were collected

before the feed delivery (0 h) and at 2, 4, 8, and 12 h after the feed delivery on d 14 of each experimental period. Ruminal pH was recorded immediately after sampling using a portable pH meter (Bel Equipamentos Analíticos, Piracicaba, SP, Brazil). Subsamples of rumen fluid (50 ml each) were stored at −20 °C without preservatives for analyses of volatile fatty acids (VFA[13] 1961) by gas chromatography (Shimadzu GC-2010; Shimadzu Corporation, Kyoto, Japan), and ruminal ammonia nitrogen (N-NH_3 [14]) by spectrophotometry (Biospectro SP-22; Biospectro, Curitiba, PR, Brazil).

Statistical analyses

Data were analyzed using the "easyanova" package[10] of R software[10] in a 5 × 5 Latin square design. Treatments and periods were included in the model as fixed effects. Steer was the experimental unit and was included in the model as a random effect according to equation: Yijk= μ + Ai + Pj + Dk + eijk, Where: Yijk= dependent variable, μ= overall mean, Ai= random effect of animal (i = 1 to 5), Pj= fixed effect of period (j = 1 to 5), Dk= fixed effect of treatment (k= 1 to 5), eijk= random error associated with the observation.

Rumen fermentation data (pH, NH_3-N, and VFA) were presented by averaging sampling time, according to the equation: Yijkl= μ + Ai + Pj + Dk + eijk + Tl + D*T + elk Where: Yijkl= dependent variable, μ= overall mean, Ai= random effect of animal (i= 1 to 5), Pj= random effect of period (j= 1 to 5), Dk= fixed effect of treatment (k= 1 to 5), eijk= type 1 error, Tl= fixed effect of time (l= 1 to 5), and elk= type 2 error. Statistical differences were declared at $P<0.05$, and trends were discussed at $P\leq0.10$.

The Tukey comparison test was used to test for differences between means ($P<0.05$). Means are presented as least squares means with SEM.

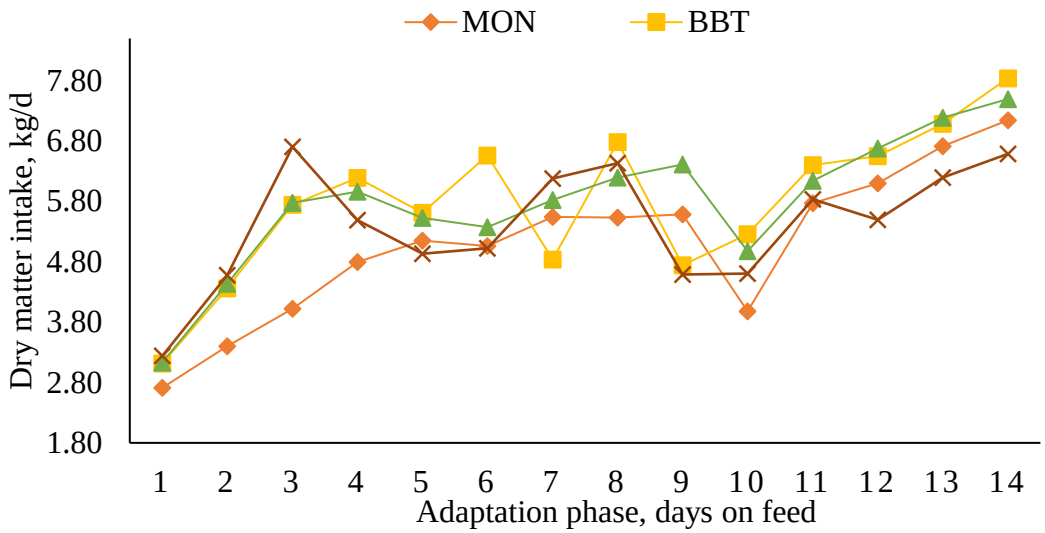
Results

Experiment 1

There was no block effect for the variables studied, and it was considered a random effect. Bulls had a continuous increase in DMI throughout the adaptation phase and reached a DMI of 6.97 kg/bull on d 14 (Figure 1; treatment $P<0.001$, SEM= 0.312; day $P<0.001$; treatment × day $P<0.001$). The overall average DMI during the adaptation phase (i.e., d 1 to 14) was

4.80, 5.54, 5.54, and 5.51 kg for MON, BBT, BEO, and BFO, respectively. Over the entire feeding period (i.e., d 1 to 104), the DMI of bulls fed MON was 16, 13, and 9 % lower than BBT, BEO, and BFO, respectively (Table 2). No changes (P>0.05) were observed on the final BW (overall mean 486 kg), ADG (overall mean 1.42 kg), and carcass characteristics (Table 2). Feed efficiency of bulls fed MON was 13 %, 11 %, and 4 % greater than BBT, BEO, and BFO, respectively (P<0.05; Table 2).

Figure 1: Dry matter intake (DMI, kg/d) of *Bos indicus* bulls throughout the adaptation phase (d 1–14) to a finishing diet (20:80, roughage [sugarcane bagasse] to concentrate ratio)



MON= 30 mg/kg [Rumensin-200); BBT= 1,500 mg/kg of extract from barbatimão [*Stryphnodendron adstringens*]; BEO= 118 mg/kg of the blend of essential oils; BFO= 250 mg/kg of the blend of functional oils.

Treatment P<0.001 (SEM= 0.312); day P<0.001; treatment × day P<0.001.

Table 2: Dry matter intake, growth performance, and carcass characteristics of *Bos indicus* bulls fed a finishing diet containing monensin and phytogenic antimicrobial feed additives

Item	Feed additive				SEM	*P*-value
	MON	BBT	BEO	BFO		
Dry matter intake:						
d 1 to 104						
kg/d	7.57[c]	9.03[a]	8.75[ab]	8.28[b]	0.127	<0.001
% of BW	1.85[c]	2.20[a]	2.13[a]	2.01[b]	0.026	<0.001
Growth performance						
Body weight, kg						
Initial (d 1)	336	336	335	335	0.2	0.358
Final (d 104)	482	485	487	489	3.9	0.599
ADG, kg/d	1.39	1.42	1.44	1.46	0.037	0.524
Feed efficiency	0.184[a]	0.160[b]	0.164[b]	0.176[ab]	0.005	0.010
Carcass characteristics:						
Hot carcass weight, kg	271.2	273.8	274.7	276.1	1.72	0.279
Dressing percentage[1]	56.2	56.5	56.4	56.4	0.28	0.942
Backfat thickness, mm	3.14	2.60	2.79	2.82	0.221	0.418

MON= 30 mg/kg of monensin (Rumensin-200; BBT= 1,500 mg/kg of extract from barbatimão (*Stryphnodendron adstringens*); BEO= 118 mg/kg of the blend of essential oils; BFO= 250 mg/kg of the blend of functional oils.

[1] Calculated dividing the hot carcass weight by the final body weight (d 104).

[a–c] Means within rows that do not have a common superscript differ ($P<0.05$).

Experiment 2

Nutrient's intake and apparent total tract digestibility did not differ among feed additives ($P>0.05$), except that digestibility of ADF tended ($P=0.084$) to be greater for MON and BFO than other additives (Table 3). The lowest and the highest pH values were observed for BBT (6.54) and BFO (6.75), respectively ($P<0.05$; Table 4). The valerate concentration was 19 % higher for BBT and BEO compared with MON ($P<0.05$). Concentrations of NH_3-N, total VFA, acetate, propionate, butyrate, isobutyrate, and isovalerate were similar among feed additives ($P>0.05$; Table 4).

Table 3: Intake and apparent total tract digestibility of nutrients from a finishing diet (roughage to concentrate ratio 20:80) containing antimicrobial feed additives fed to *Bos indicus* steers

Item	Feed additive[1]					SEM	*P*-value
	CTL	MON	BBT	BEO	BFO		
Intake, kg/d							
Dry matter	6.51	7.04	7.12	5.94	7.21	0.713	0.559
Organic matter	6.28	6.78	6.86	5.73	6.96	0.685	0.558
Crude protein	1.01	1.06	1.20	0.98	1.12	0.116	0.619
NDF	2.29	2.52	2.45	2.12	2.60	0.245	0.530
ADF	1.27	1.38	1.33	1.12	1.42	0.143	0.460
Hemicellulose	1.02	1.14	1.12	1.01	1.17	0.118	0.711
Fecal output							
kg DM/d	2.74	2.07	2.50	2.46	2.53	0.217	0.234
Digestibility, g/g							
Dry matter	0.57	0.69	0.64	0.57	0.65	0.041	0.166
Organic matter	0.59	0.71	0.66	0.60	0.67	0.039	0.162
Crude protein	0.76	0.78	0.78	0.75	0.76	0.027	0.771
NDF	0.49	0.60	0.55	0.49	0.60	0.063	0.437
ADF	0.43	0.58	0.51	0.42	0.57	0.051	0.084
Hemicellulose	0.56	0.63	0.60	0.56	0.63	0.090	0.924

CTL= no additive; MON= 30 mg/kg of monensin; BBT= 1,500 mg/kg of extract from barbatimão (*Stryphnodendron adstringens*); BEO= 118 mg/kg of the blend of essential oils and pepper extract; BFO= 250 mg/kg of the blend of functional oils.

Table 4: Rumen fermentation characteristics of *Bos indicus* steers fed a finishing diet (roughage to concentrate ratio 20:80) containing antimicrobial feed additives

Item	Feed additive					SEM	*P*-value
	CTL	MON	BBT	BEO	BFO		
Rumen, pH	6.69[ab]	6.59[ab]	6.54[b]	6.66[ab]	6.75[a]	0.050	0.042
NH_3-N, mg/dl	13.62	10.17	11.88	12.59	11.51	1.943	0.713
VFA, mM							
Total	38.20	42.72	52.88	51.97	47.98	6.199	0.234
Acetate (C_2)	24.10	27.26	34.63	33.87	30.74	4.144	0.297
Propionate (C_3)	7.33	9.46	9.95	10.63	9.85	1.728	0.685
C_2:C_3	3.46	3.13	3.53	3.31	3.46	0.139	0.225
Butyrate	4.58	4.64	6.04	5.30	5.24	0.507	0.200
Isobutyrate	0.51	0.47	0.56	0.52	0.51	0.025	0.168
Valerate	0.78[ab]	0.68[b]	0.81[a]	0.81[a]	0.72[ab]	0.028	0.009
Isovalerate	0.89	0.84	0.88	0.85	0.83	0.036	0.707

CTL= no additive; MON= 30 mg/kg of monensin; BBT= 1,500 mg/kg of extract from barbatimão (*Stryphnodendron adstringens*); BEO= 118 mg/kg of the blend of essential oils and pepper extract; BFO= 250 mg/kg of the blend of functional oils.

[a–b] Means within rows that do not have a common superscript differ ($P<0.05$).

Discussion

A control diet with no additives was not included in the growth performance experiment due to concerns that cattle not supplemented with antimicrobial feed additives would be exposed to the risk of health issues associated with acidosis (e.g., rumenitis, parakeratosis, liver abscesses, laminitis, and polioencephalomalacia). Ionophores enhance feed efficiency and reduce the incidence of digestive disorders, such as acidosis, in ruminants[15]. Monensin is an ionophore widely studied and used in animal production and was considered suitable to test the hypothesis of the current study.

The continuous increase in DMI throughout d 1 to 14 on feed indicates that the adaptation protocol was adequate, and bulls seemed to be adapted to the finishing diet. On d 14, bulls had reached 83 % (6.97 kg/d) of the overall average DMI of the entire feeding period (8.41 kg/d). The satisfactory increase in DMI is particularly critical because responses observed in the adaptation phase may impose long-term consequences and affect the cattle growth performance through the end of the experiment. The reduced DMI of bulls fed MON observed over the entire feeding period (i.e., d 1 to 104) can be attributed to the biological

actions of ionophores (e.g., improved energy and nitrogen metabolism), which have been well-documented in previous research of phytogenic additives compared with monensin[16,17,18]. However, previous research had shown no changes in DMI of finishing cattle when monensin was compared with condensed tannins[19], a blend of essential oils similar to the current study[20], and a mix of functional oils (castor oil and cashew nut shell liquid[21,22]).

Similar responses observed on ADG, final BW, and carcass characteristics, which were also reported by Araujo *et al*[20] in a study with MON and BEO (n= 656 steers), are evidence that naturally-occurring compounds present in the phytogenic products represent safe alternatives to replace antibiotics currently used in animal nutrition. However, the least DMI of cattle fed MON led to the highest feed efficiency in the current study. It also was observed when MON was compared with a differing essential oil mixture (thymol, eugenol, vanillin, guaiacol, and limonene[22]) and functional oils (castor oil and cashew nut shell liquid[20]).

Feed additives did not change DMI in the metabolism trial (i.e., Exp. 2). The overall average DMI was lower in Exp. 2 than values recorded for bulls in the growth performance trial (6.85 *vs* 8.41 kg/d, respectively). Numerous aspects, such as the experimental design (Latin square *vs.* randomized block design), housing (individually *vs* group), sexual class (steers *vs* bulls), and initial body weight (275 *vs* 336 kg), would have accounted for the discrepancy in DMI between trials. Similar to this study, the absence of changes in apparent digestibility of nutrients was reported for MON compared with mixtures of essential oils by Benchaar *et al*[16] (thymol, eugenol, vanillin, and limonene) and Meyer *et al*[21] (thymol, eugenol, vanillin, guaiacol, and limonene). Possible causes to explain this response are unclear. The absence of effects on apparent total tract digestibility of nutrients, however, could have reflected a large inter-animal and day-to-day variations.

Ruminal pH ranges from 5.8 to 6.5 in grain-adapted cattle, and values of 5.6 and 5.0 are benchmarks for chronic (i.e., subacute) and acute acidosis, respectively[23]. The relatively high inclusion level of roughage (i.e., 200 g/kg DM of sugarcane bagasse) in the finishing diet might have contributed to maintain ruminal pH above 6.5 and avoid digestive disorders. Sugarcane bagasse contains 758 ± 112 g/kg NDF (NASEM[7]; 837 g/kg NDF in the current study), and an average particle size of 13.5 mm and particle size distribution of 70 % > 8.0 mm using the Penn State Particle Separator[24].

The lowest pH value was observed for BBT (6.54), suggesting that cattle might have not experienced acidosis. Such response could be due to ciliate protozoa inhibition by condensed tannins[25], although it was not formally measured in the present study. Rumen protozoa engulf starch particles and store glucose as polysaccharides, delaying starch fermentation by bacteria, which retard acid production and stabilize ruminal fermentation[26]. Tannins act directly on microbial metabolism through interactions with the cell wall and indirectly by

inhibiting microbial enzymes or by binding to dietary components such as protein and carbohydrates, preventing the degradation of substrates by rumen microorganisms[27]. However, rumen protozoa are also sensitive to monensin[16,28] and functional oils such as cashew nut shell liquid[29], and a blend of castor oil with cashew nut shell liquid[22]. Rumen protozoa seemed not to be sensitive to a mix of essential oils similar to the current study, which was supplemented at a slightly higher level (150 *vs* 118 mg/kg DM in the current study) to dairy cows fed a diet with 51:49 roughage to concentrate ratio[30]. A meta-analysis conducted by Khiaosa-ard and Zebeli[31] showed that only high doses (>200 mg/kg DM) of essential oils and their bioactive compounds had an inhibitory effect in rumen protozoa.

Because the rumen is a highly complex and competitive environment, inhibiting Gram-positive bacteria leads to the proliferation of Gram-negative bacteria, which are more prone to produce propionate, changing the proportions of ruminal VFA and other fermentation products[28]. Gram-positive bacteria (e.g., hyper-ammonia-producing and cellulolytic bacteria) are proven to be sensitive to monensin[28] and plant extracts such as condensed tannins[25,32]), a blend of essential oils[33], a blend of the functional oils castor oil and cashew nut shell liquid[22], and cashew nut shell liquid[1] and copaíba[34] oils individually. However, no changes were observed in the current study on the concentration of NH_3-N and VFA, except for valerate. The antimicrobial activity of many plant extracts might be pH-dependent. The low pH (5.5) increases the proportion of undissociated, hydrophobic forms of the active molecules (enhancing the interactions with bacterial cell walls) and the susceptibility of specific microbial populations such as Gram-positive bacteria. Cardozo *et al*[35]reported no beneficial changes in rumen fermentation at pH 7.0 (overall mean was 6.64 in the current experiment).

Valeric acid is mainly built up from the catabolism products of the amino acid proline, and it enhances cellulose digestion by rumen microorganisms[36,37]. The causes of the increase in valerate with no changes in the digestibility of NDF observed for BBT and BEO are unclear. Information regarding the intermediate products formed in the catabolism of condensed tannins and specific essential oils by rumen microorganisms is still scant. Unlike the findings reported in the current study, Castillejos *et al*[38] noted that monensin increased the concentration of valerate compared with essential oils when evaluating a 10:90 (roughage to concentrate ratio) diet. There is no evidence in the literature that the degradation of tannins and essential oils could drive the increase in valerate concentration. Furthermore, inconsistencies within the literature and between responses observed in the current study and previous researches likely reflect the variable nature of phytogenic products themselves, supplementation levels, experimental conditions, as well as the complexity of possible interactions of the rumen microbiome with bioactive compounds and feed components.

Conclusions and implications

In management involving the use of monensin, better feed efficiency was observed. Therefore, even generating carcass values similar to other management practices involving the use of other additives, the use of monensin can optimize the production of high-value products, such as meat.

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Competing interests

The authors declare there are no conflicts of interest.

Literature cited:

1. Oh S, Suzuki Y, Hayashi S, Koike S, Kobayashi Y. Potency of cashew nut shell liquid in rumen modulation under different dietary conditions and indication of its surfactant action against rumen bacteria. J Anim Sci Technol 2017;59-27.

2. Reddy PRK, Elghandour MMMY, Salem AZM, Yasaswini D, Reddy PPR, Reddy AN, Hyder I. Plant secondary metabolites as feed additives in calves for antimicrobial stewardship. Anim Feed Sci Technol 2020;264:114469.

3. Hagerman AE, Butler LG. Protein precipitation method for the quantitative determination of tannins. J Agric Food Chem 1978;26:809-812.

4. Lemos BJM, Souza FM, Arnhold E, Conceição EC, Couto VRM, Fernandes JJR. Effects of plant extracts from *Stryphnodendron adstringens* (Mart.) Coville, *Lafoensia pacari* A. St.-Hil., *Copaifera* spp., and *Pterodon emarginatus* Vogel on *in vitro* rumen fermentation. J Anim Physiol Anim Nutr 2021;00:1-14.

5. Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medicines 2015;2:251-286.

6. Murakami AE, Eyng C, Torrent J. Effects of functional oils on coccidiosis and apparent metabolizable energy in broiler chickens. Asian-Australas J Anim Sci 2014;27:981-989.

7. Sousa JN, Pedroso NB, Borges LL, Oliveira GAR, Paula JR, Conceição EC. Optimization of ultrasound-assisted extraction of polyphenols, tannins and epigallocatechin gallate from barks of *Stryphnodendron adstringens* (Mart.) Coville bark extracts. Pharmacogn Mag 2014;10:S318–S323.

8. NASEM (National Academies of Sciences, Engineering, and Medicine). Nutrient requirements of beef cattle. 8th ed. Washington: National Academies Press; 2016.

9 AOAC. Official Methods of Analysis of AOAC International (OMA). Gaithersburg: AOAC International; 2005.

10. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583-3597.

11. Arnhold E. Package in the R environment for analysis of variance and complementary analyses. Braz J Vet Res Anim Sci 2013;50:488-492.

12. R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2019.

13. Williams CH, David DJ, Iismaa O. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J Agric Sci 1962;59:381-385.

14. Erwin ES, Marco GJ, Emery EM. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J Dairy Sci 1961;44:1768-1771.

15. Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. Clin Chem 1962;8:130-132.

16. Bergen WG, Bates DG. Ionophores: their effect on production efficiency and mode of action. J Anim Sci 1984;58:1465-1483.

17. Benchaar C, Duynisveld JL, Charmley E. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. Can J Anim Sci 2006;86:91-96.

18. Fandiño I, Calsamiglia S, Ferret A, Blanch M. Anise and capsicum as alternatives to monensin to modify rumen fermentation in beef heifers fed a high concentrate diet. Anim Feed Sci Technol 2008;145:409-417.

19. Wu J, Bai Y, Lang X, Wang C, Shi X, Casper DP, Zhang L, Liu H, Liu T, Gong X, Liang T, Zhang R. Dietary supplementation with oregano essential oil and monensin in combination is antagonistic to growth performance of yearling Holstein bulls. J Dairy Sci 2020;103:8119-8129.

20. Araujo RC, Daley DR, Goodall SR, Jalali S, Guimarães Bisneto OA, Budde AM, *et al*. Effects of a microencapsulated blend of essential oils supplemented alone or in combination with monensin on performance and carcass characteristics of growing and finishing beef steers. Appl Anim Sci 2019;35:177-184.

21. Purevjav T, Hoffman MP, Ishdorj A, Conover AJ, Jedlicka ME, Prusa K, Torrent J, Pusillo GM. Effects of functional oils and monensin on cattle finishing programs. Prof Anim Sci 2013;29:426-434.

22. Meyer NF, Erickson GE, Klopfenstein TJ, Greenquist MA, Luebbe MK, Williams P, Engstrom MA. Effect of essential oils, tylosin, and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation, and digestibility. J Anim Sci 2009;87:2346-2354.

23. Zotti CA, Silva AP, Carvalho R, Marino CT, Rodrigues PHM, Silva LFP, *et al*. Monensin and a blend of castor oil and cashew nut shell liquid used in a high-concentrate diet abruptly fed to Nellore cattle. J Anim Sci 2017;95:4124-4138.

24. Nagaraja TG, Titgemeyer EC. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. J Dairy Sci 2007;90:(E.Suppl.).

25. Marques RS, Chagas LJ, Owens FN, Santos FAP. Effects of various roughage levels with whole flint corn grain on performance of finishing cattle. J Anim Sci 2016;94:339-348.

26. Vasta V, Daghio M, Cappucci A, Buccioni A, Serra A, Viti C, Mele M. Invited review: plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: experimental evidence and methodological approaches. J Dairy Sci 2019;102:3781-3804.

27. Huntington GB. Starch utilization by ruminants: from basics to the bunk. J Anim Sci 1997;75:852-867.

28. Scalbert A. Antimicrobial properties of tannins. Phytochem 1991;30:3875-3883.

29. Russel JB, Houlihan AJ. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiol Rev 2003;27:65-74.

30. Watanabe Y, Suzuki R, Koike S, Nagashima K, Mochizuki M, Forster RJ, Kobayashi Y. *In vitro* evaluation of cashew nut shell liquid as a methane-inhibiting and propionate-enhancing agent for ruminants. J Dairy Sci 2010;93:5258-5267.

31. Silva RB, Pereira MN, Araujo RC, Silva WR, Pereira RAN. A blend of essential oils improved feed efficiency and affected ruminal and systemic variables of dairy cows. Transl Anim Sci 2020;4:182-193.

32. Khiaosa-ard R, Zebeli Q. Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants. J Anim Sci 2013;91:1819-1830.

33. Waghorn G. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. Anim Feed Sci Technol 2008;147:116-139.

34. McIntosh FM, Williams P, Losa R, Wallace RJ, Beever DA, Newbold CJ. Effects of essential oils on ruminal microorganisms and their protein metabolism. Appl Environ Microbiol 2003;69:5011-5014.

35. Santos AO, Ueda-Nakamura T, Dias Filho BP, Veiga Jr VF, Pinto AC, Nakamura CV. Antimicrobial activity of Brazilian copaíba oils obtained from different species of the Copaifera genus. Mem Inst Oswaldo Cruz 2008;103:277-281.

36. Cardozo PW, Calsamiglia S, Ferret A, Kamel C. Screening for the effects of natural plant extracts at different pH on *in vitro* rumen microbial fermentation of a high-concentrate diet for beef cattle. J Anim Sci 2005;83:2572-2579

36. Dehority BA, Johnson RR, Bentley OG, Moxon AL. Studies on the metabolism of valine, proline, leucine and isoleucine by rumen microorganisms *in vitro*. Arch Biochem Biophys 1958;78:15-27.

37. Amos HE, Little CO, Mitchell GE. Proline utilization during cellulose fermentation by rumen microorganisms. J Agric Food Chem 1971;19:112-115.

38. Castillejos L, Calsamiglia S, Martín-Tereso J, Wijlen HT. *In vitro* evaluation of effects of ten essential oils at three doses on ruminal fermentation of high concentrate feedlot-type diets. Anim Feed Sci Technol 2008;145:259-270.