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Monensin and a blend of castor oil and cashew nut shell liquid used in a high-concentrate diet abruptly fed to Nellore cattle[1]

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ABSTRACT: Monensin and functional oils (FO) were supplemented to a high-concentrate diet abruptly fed to 12 ruminally cannulated Zebu steers to study their effects on rumen fermentation, blood metabolites, and *Streptococcus bovis*, *Megasphaera elsdenii*, and *Fibrobacter succinogenes* relative population. A randomized complete block design with repeated measures over time within 2 experimental periods of 21 d each was used. Treatments were a control (CTR; with no additives), FO (included at 400 mg/kg), and monensin included at 30 mg/kg (M30) or 40 mg/kg (M40). All steers were fed the same high-concentrate basal diet, which consisted of 92.25% concentrate. The first 60 h after transition showed a treatment and hour interaction for ruminal propionate proportion ($P = 0.028$), and no change in acetate molar proportion ($P = 0.633$), rumen pH ($P = 0.370$), and time the rumen pH remained below 5.6 ($P = 0.242$) were observed. The acetate:propionate ratio decreased ($P = 0.020$) when monensin was fed in both concentrations (2.30 for the M30 treatment and 2.32 for the M40 treatment) compared with when the CTR was fed (2.85), without being different when the FO (2.71) treatment was fed. Only the M30 treatment did not show pH below 5.2 (P=0.047) over the 60 h after the abrupt transition. Within the entire period, DMI ($P = 0.008$) and mean ruminal pH ($P = 0.040$) as well as molar proportions of propionate ($P = 0.034$) and valerate ($P = 0.031$) had significant interactions between treatment and day. Total VFA concentration was greater ($P = 0.017$) for the M30 (117.36 m*M*) and CTR treatments (115.77 m*M*) compared with the M40 treatment (105.02 m*M*), without being different for the FO treatment (111.55 m*M*). Treatments did not change feed behavior parameters. Blood HCO_3^- ($P = 0.006$) and total carbon dioxide ($P = 0.003$) were greater for the M30 (27.8 and 29.3 mmol/L, respectively) and FO treatments (28.3 and 29.7 mmol/L, respectively) compared with the CTR treatment (25.7 and 26.9 mmol/L, respectively). *Fibrobacter succinogenes* ($P < 0.0001$) and *Streptococcus bovis* ($P < 0.0001$) decreased their population throughout days, whereas *Megasphaera elsdenii* ($P = 0.026$) increased its population. Independent of ciliated protozoa genera, the greatest ($P < 0.0001$) protozoa counts were observed for the CTR treatment (52.7×10^4/mL), intermediate for the FO treatment (35.3 x10^4/mL), and least for steers fed monensin in both concentrations (15×10^4/mL for the M30 treatment and 14×10^4/mL for the M40 treatment). Feed additives had different effects to reduce the subacute acidosis. The use of the FO and M40 treatments did not change most of the rumen fermentation variables, especially in the first week after abrupt transition, when the M30 treatment provided higher protection against acidosis.

Key words: abrupt challenge, beef cattle, feed additives, rumen metabolism, ruminal acidosis

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INTRODUCTION

Ruminal acidosis is considered the most common nutritional disorder in feedlot cattle fed rapidly fermentable nonstructural carbohydrates (Nagaraja

and Titgemeyer, 2007). Monensin, an ionophore antibiotic, has been reported to reduce DMI variation and pH change (Erickson et al., 2003); to modify rumen metabolism (decrease acetate:propionate ratio), leading to fewer acidosis episodes; and to improve feed:gain ratio (Nagaraja and Lechtenberg, 2007). Natural alternatives such as functional oils (**FO**) that increase the propionate and decrease acetate, methane, and proteolysis production are highly desired (Calsamiglia et al., 2007) and might be used as ruminal modifiers with the potential to replace antibiotics (Benchaar et al., 2008). Functional oils are defined as those oils that have an action beyond the nutritional value and do not derive from essences and spices (Murakami et al., 2014), whereas essential oils are naturally occurring volatile components responsible for the characteristics of essence and color of plants (Benchaar et al., 2008). Castor oil acid and cashew nut shell liquid have ricinoleic and anacardic acids and cardanol and cardol as the main components. These components exhibited antimicrobial activity, especially the fatty acids (approximately 90% ricinoleic acid) in castor oil, which has been described as inhibitor of biohydrogenation and methane production (Morales et al., 2012), and also with action on some Gram-positive bacteria (Novak et al., 1961). An in vivo study showed the potential of FO as a feed additive, where although steers in this group had similar ADG and lower G:F, they had also higher percent of prime and choice quality grade, which positively influenced profitability, when compared with steers fed diets supplemented with monensin (Purevjav et al., 2013). Rapid introduction of a finishing diet to beef cattle is necessary to improve ADG and feed:gain ratio. Few studies have assessed the effect of abrupt transition (Burrin and Britton, 1986), and little is known about abrupt high-concentrate feed system on Zebu beef cattle. The hypothesis of the present study was that the addition of different feed additives may modulate ruminal fermentation toward a reduction in acidosis experienced when steers were abruptly fed a high-concentrate diet. The objective was to determine whether monensin concentration and a blend of FO favorably affected the rumen metabolism and feed behavior of Nellore steers.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Care Committee of the University of Sao Paulo.

Animals, Housing, and Diets

This trial was performed at the metabolism barn of the College of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil. Twelve ruminally cannulated Nellore steers (532 ± 14 kg BW) were used in 2 measurement periods of 21 d. The experiment was designed as a randomized complete block (period). In each period, 3 animals were assigned in to 1 of 4 groups. For 6 wk before the start of the experiment and between the 21-d experimental periods, all steers were allowed ad libitum access to *Cynodon dactylon* Tifton 85 hay. On the transition day (d 1), a basal diet (Table 1) was abruptly fed to the animals at 1.8% of BW; thereafter, the feed was offered ad libitum over 21 d. Additives were mixed into the concentrate and then to the basal diet. The basal diet was delivered as a total mixed ration once daily at 0700 h, and steers had free access to water. The total mixed ration was formulated according to NRC (1996) recommendations. The treatments consisted of a basal diet without additives, the control (**CTR**), and to this basal diet, FO fed at 400 mg/kg and monensin included at 30 mg/kg (**M30**) and 40 mg/kg (**M40**) were included. The FO was a blend of castor oil acid and cashew nut shell liquid, containing ricinoleic acid, anacardic acid, cardanol, and cardol as active principles (Oligo Basics Agroindustrial Ltda., Cascavel, Paraná, Brazil). Monensin dose (M30) was chosen to simulate practical conditions adopted in feedlots, whereas the M40 was chosen to provide less metabolic challenge to animals, as a reduction in feed intake is expected with this treatment. For the FO treatment, the dose adopted was based on results described by Coneglian (2009).

Table 1. Ingredients proportion and nutrient composition of the basal diet[1] (DM basis)

Item	Percent
Ingredient	
Cracked corn	82.4
Tifton 85 bermudagrass hay	7.75
Soybean meal	6.78
Urea	1.29
Calcitic lime (calcium carbonate)	0.71
Potassium chloride	0.53
Mineral premix[2]	0.50
Chemical composition	
DM, %	82.0
CP, % of DM	16.7
NDF, % of DM	16.6
ADF, % of DM	7.68
Ether extract, % of DM	4.00
Starch, % of DM	54.5
TDN,[3] %	87.2

[1]Diet was formulated according to the NRC (1996).

[2]Mineral premix included 1,000 mg/kg manganese, 2,000 mg/kg zinc, 10 g/kg magnesium, 560 mg/kg cupper, 30 mg/kg iodine, 15 mg/kg cobalt, 6 mg/kg selenium, 30 g/kg sulfur, 60 g/kg sodium, and 100 g/kg potassium.

[3]Estimated according the equation described by Weiss et al. (1992)

Feed Intake and Animal Feed Behavior

Individual DMI was assessed from d 4 (before abrupt transition) to 21 (after abrupt transition) and calculated as the difference between the DM offered and the DM of orts. Animal feed behavior was recorded by a color micro-camera (model 420 TVL; Sony Corporation, Tokyo, Japan) for 24 h during d 1, 2, 3, 12, and 20 of each experimental period. Feeding behavior was analyzed as described by Bingham et al. (2009). Briefly, individual head-down feeding events (events/d) were registered; a new event started when the steers lowered their head to ingest feed and ended when the steers raised their head above the feed. The total duration (min) of each head-down event was registered and presented as minutes per day. Rate of ingestion was calculated from the daily DMI (g) divided by the total head-down duration (min). Using this method, we registered the entire time that each steer spent eating at the feeding bunk.

Chemical Analyses

Feed offered and orts were sampled daily, pooled for each period and steer, and dried in forced-air oven at 55°C for 48 h. Samples were ground (1-mm screen) using a hammer mill (model TE-651/2; Tecnal, Piracicaba, Sao Paulo, Brazil). Analytical DM was analyzed according to the Association of Official Analytical Chemists (method 934.01; AOAC, 1990). Nitrogen content was determined by a micro-Kjeldahl method (method 920.87; AOAC, 1990), and the CP was calculated (nitrogen × 6.25). Neutral detergent fiber and ADF were determined according to Van Soest et al. (1991).

Indwelling pH Measurement

Ruminal pH was continuously measured by indwelling pH probes (model T7-1 LRCpH; Dascor, Escondido, CA). Standard solutions of pH 7 and 4 were used for calibrate the probe pH meter as described by Penner et al. (2006). Rumen pH data was recorded with 15-min intervals from d 1 to 21, and data were summarized for each steer as the daily mean pH and minimum and maximum pH. The duration under pH thresholds 5.6 and 5.2 as well as the area under the curve were calculated as described by Moya et al. (2011). The durations under pH thresholds 5.6 and 5.2 were used as subacute and acute acidosis criteria, respectively (Bevans et al., 2005). Area under the curve of pH 5.6 and 5.2 was used to indicate the severity of subacute and acute acidosis, respectively.

Rumen Sampling

During the first 3 d after the abrupt transition, rumen fluid samples were taken at 0, 6, 12, 24, 30, 36, 48, 54, and 60 h after feeding. Within each period (21 d), rumen fluid samples were taken on d 1, 2, 3, 5, 7, 10, 13, 17 and 21; 6 h post-feeding. Rumen fluid samples of approximately 200 mL were taken from different locations in the rumen, using an electric vacuum pump. Then, the rumen fluid was squeezed through 4 layers of cheesecloth. Four subsamples of 15 mL each were pipetted into micro tubes and frozen at −20°C until analysis. After thawing, VFA samples were centrifuged at 18,000 × g for 10 min at 4ºC, and 800 µL of supernatant was mixed with 200 µL of formic acid and 100 µL of internal standard (2- ethylbutyric acid). Analysis of the VFA, which included acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate, was performed on a gas chromatograph (Shimadzu GC-2014; Shimadzu Corp., Kyoto, Japan) equipped with a capillary column (Stabilwax; 30 m length and 0.53 mm internal diameter). The injector and flame ionization detector were at 250°C and He was used as carrier gas (8.01 mL/min flow). Before freezing, NH_3–N samples were acidified with 1 mL of 1 *N* sulfuric acid and analyzed according to Weatherburn (1967). Samples for lactic acid analyses were centrifuged at 15,000 × g for 15 min at room temperature, and the supernatant was analyzed as described by Pryce (1969) using spectrophotometry (Nova 2000 UV; Nova Instruments, Piracicaba, Sao Paulo, Brazil) at 565 nm. Ruminal fluid osmolality was analyzed by freezing point depression using the Advanced Micro-Osmometer (model 3300; Advanced Instruments, Inc., Norwood, MA).

Rumen Protozoa

On d 5 and 21, rumen fluid samples were taken 6 h after feeding to count the ciliate protozoa genera (*Entodinium*, *Diplodinium*, *Epidinium*, *Isotricha*, *Dasytricha*, *Ostracodinium*, *Eudiplodinium*, and *Enoploplastron* spp.). Solid rumen digesta was taken from several locations in the rumen and squeezed through 3 layers of cheesecloth. Thereafter, 10 mL of filtered fluid was pipetted into tubes containing 20 mL of formaldehyde solution at 37% (vol/vol). Then, samples were placed into a counting chamber (Sedgewick Rafter; Pyser-SGI, Kent, UK), and ciliate genera were identified as described Dehority (2003).

Real-Time PCR of Rumen Bacteria

Rumen samples for PCR were taken on d −1 (baseline), 2, 5, 10, and 21 of each period at 6 h after feeding. Fluid and particulate samples were collected from different rumen locations (cranial and dorsal in the middle, bottom, and top), mixed, and separated into 2 aliquots of 25 mL. The sample processing was performed as described by Stevenson and Weimer (2007). Thereafter,

Table 2. Specific primers used for the quantification of bacteria by real-time PCR

Microorganism	16S RNA primers[1]	Reference
Total bacteria	F: GTGSTGCAYGGYTGTCGTCA	Maeda et al. (2003)
	R: ACGTCRTCCMCACCTTCTC	
Fibrobacter succinogenes	F: GGTATGGGATGAGCTTGC	Koike and Kobayashi (2001)
	R: GCCTGCCCCTGAACTATC	
Megasphaera elsdenii	F: GACCGAAACTGCGATGCTAGA	Ouwerkerk et al. (2002)
	R: TCCAGAAAGCCGCTTTCGCCACT	
Streptococcus bovis	F: TTCCTAGAGATAGGAAGTTTCTTCGG	Stevenson and Weimer (2007)
	R: ATGATGGCAACTAACAATAGGGGT	

[1]F = forward; R = reverse.

the bacteria pellet was dissolved in 700 µL of buffer and kept at −80°C until DNA extraction. Duplicates of 100 flow µL of each ruminal sample were used for DNA extractions, which were performed using a Qiagen DNA stool mini kit (QIAGEN GmbH, Hilden, Germany) according to de Souza et al. (2017). Real-time PCR was performed with ABI PRISM 7500 (Applied Biosystems, Foster City, CA) using 96-well plates in duplicate and using water as a negative control. In each reaction mixture, 1x of SYBR Green (Applied Biosystems), 300 η*M* of each primer, 6.6 µL of nuclease-free water, and 1 µL of DNA template were used, totaling 24 µL. Primers sequences are presented in Table 2. The real-time PCR amplification cycle included an initial denaturation step at 95°C for 10 min followed by 44 cycles of heating and cooling at 95°C for 15 s and 60°C for 30 s and extension at 72°C for 30 s. Melting curve analysis was used to evaluate the amplicon specificity. According to the reaction efficiency analysis proposed by Yuan et al. (2006), all primers functioned with an efficiency not different from 100%. The relative quantification of target bacteria populations to a reference sample represented by the CTR treatment was assessed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The delta threshold cycle was first calculated subtracting the threshold cycle obtained for Eubacteria and the targeted rumen bacteria, and the delta-delta threshold cycle was then calculated considering the CTR treatment as the reference sample for each rumen bacteria.

Blood Analyses

Blood samples were taken in gel separator tubes (SST II; Becton, Dickinson and Company, Franklin Lakes, NJ) and blood gas syringes (Monovette; Sarstedt AG & Co., Nümbrecht, Germany) from the jugular vein 6 h after feeding on d 2, 3, 5, 10, and 21 of each period. Blood from separator tubes were centrifuged (1,000 × *g* for 15 min at room temperature) within 1 h, and 1 mL of serum was stored at −20°C until analysis. Serum osmolality samples were determined by freezing point depression using an Advanced Micro-Osmometer (model 3300). After blood gas collection, each sample was carefully injected into a blood-sampling cartridge (model CG4+; Abbott Point of Care Inc., Princeton, NJ.), which was plugged in a portable blood gas analyzer (i-STAT; Abbott Point of Care Inc.) The blood gas variables determined were pH, partial pressure of carbon dioxide (**pCO_2**), partial pressure of oxygen (**pO_2**), total carbon dioxide (**tCO_2**), bicarbonate (HCO_3^-), base excess (**BE**), oxygen saturation (**sO_2**), and lactate. Samples for packed cell volume (**PCV**) were collected in 10-mL Vacutainer EDTA tubes (Becton, Dickinson and Company) and PCV was determined in a hematology blood analyzer (pocH-100iV Diff; Sysmex Corp., Kobe, Japan) from whole blood (microsamples of 15 µL).

Statistical Procedures

Each steer receiving a treatment within a period was considered an experimental unit in all analyses. Data were analyzed using mixed model procedures (SAS Inst. Inc., Cary, NC), with days or hours after feeding (6, 12, 24, 30, 36, 48, 54, and 60 h) as repeated measures. The most frequent covariance structure that provided the best fit to the model was heterogeneous compound symmetry, which was determined by the lowest corrected Akaike information criteria value (Wang and Goonewardene, 2004). The error term for each of the analyzed variables was steer nested within treatment and period. For all variables analyzed, the model included fixed effects of treatment, time (days), and their interaction, and period was used as a random effect. Fixed effect of hours after feeding was included in the same model for all variables analyzed, except DMI and feed behavior variables. To reach normality, some variables were transformed by $\log_{10}$ (protozoa; time spent below pH 5.6 and 5.2) and root square (area under pH 6.0 and 5.6). Time spent below pH 5.2 and area under pH 5.2 did not achieve normality; therefore, data were submitted to a nonparametric statistical test (Kruskal–Wallis). Means among treatments were compared by Tukey test and discussed as significant effects at $P \leq 0.05$. Effect of time was discussed only when the treatment × time interaction was significant ($P \leq 0.05$).

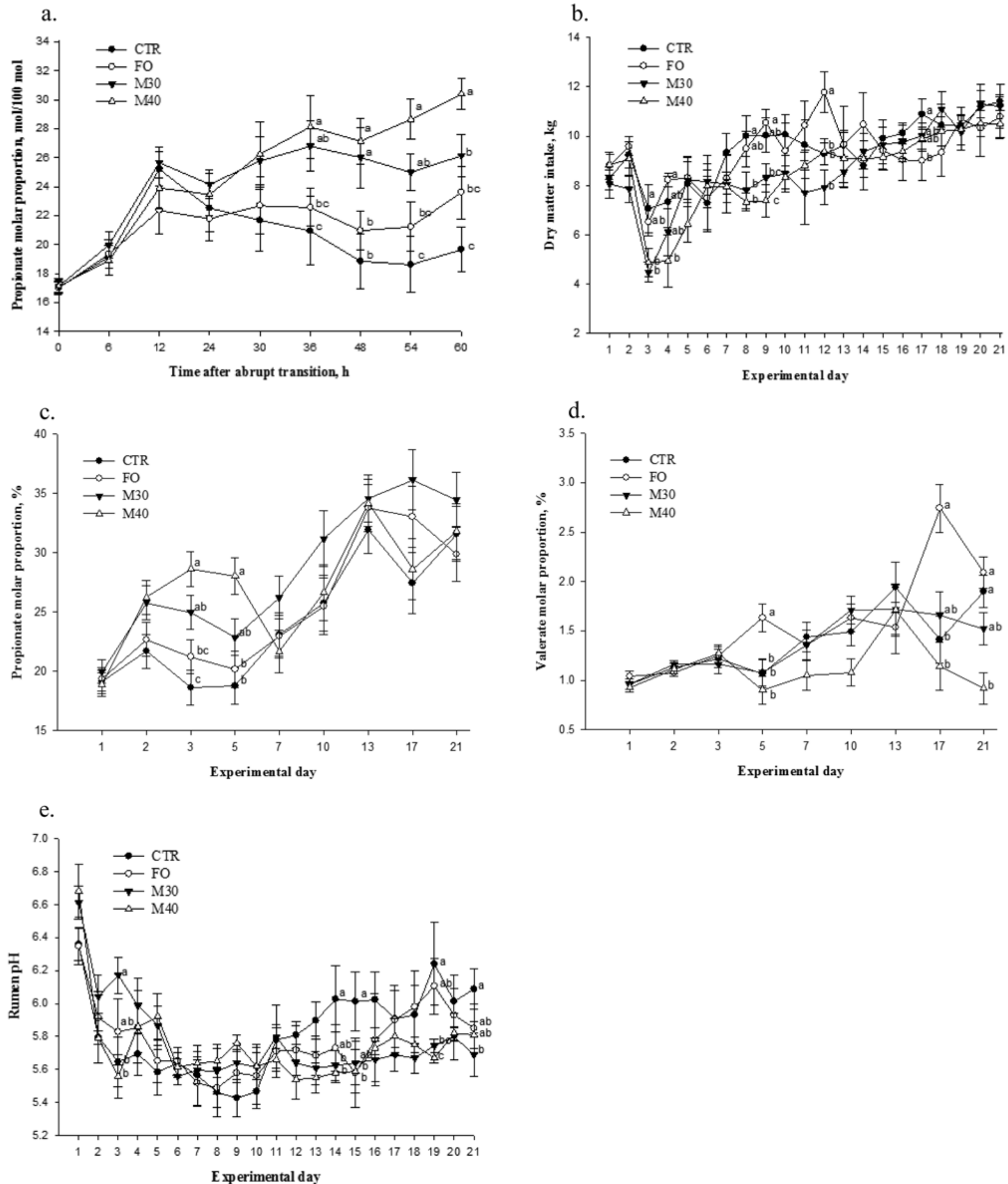

Figure 1. Propionate molar proportion during the first 60 h after abrupt transition (a), DMI (b), propionate molar proportion (c), valerate molar proportion (d), and rumen pH (e) of Nellore steers abruptly fed a high-concentrate diet with different feed additives. CTR = control (no feed additives); FO = functional oils (a blend of castor oil acid and cashew nut shell liquid fed at 400 mg/kg); M30 = monensin included at 30 mg/kg; M40 = monensin included at 40 mg/kg. [a–c]Means with different superscripts are statistically different by Tukey test ($P \leq 0.05$). Bars indicate SEM.

RESULTS

The purpose of this study in adding a blend of FO was to assess the potential of this feed additive to replace the monensin supplementation. Additionally, it is expect that as monensin concentration increases in the diet, it will provide greater protection against ruminal nutritional disorders during the first week of an abruptly fed high-concentrate diet.

Table 3. Rumen fluid fermentation patterns of Nellore steers at 60 h after abrupt transition to a high-concentrate diet[1] with different feed additives

Item	Treatments[2]				SEM	P-value[3]		
	CTR	FO	M30	M40		Trt	Hr[4]	Trt × Hr
Total VFA, mM	121.12	113.49	117.33	109.08	1.72	0.26	<0.0001	0.87
Acetate, mol/100 mol	57.04	57.16	55.41	56.62	0.46	0.63	<0.0001	0.937
Propionate, mol/100 mol	20.82	21.81	24.93	25.85	0.34	0.005	0.002	0.028
Butyrate, mol/100 mol	17.97[a]	16.82[ab]	14.86[ab]	13.14[b]	0.41	0.039	0.0008	0.124
Isobutyrate, mol/100 mol	1.13	1.22	1.13	1.11	0.03	0.73	0.0003	0.492
Valerate, mol/100 mol	1.07	1.13	1.17	1.13	0.02	0.62	0.0085	0.768
Isovalerate, mol/100 mol	2.00[ab]	1.89[b]	2.31[a]	2.18[ab]	0.05	0.021	0.0012	0.257
A:P[5] ratio	2.85[a]	2.71[ab]	2.30[b]	2.32[b]	0.05	0.020	<0.0001	0.109
NH_3–N, mg/dL	21.89	20.52	20.53	21.44	0.67	0.91	<0.0001	0.120
Lactate, mM	0.517	0.450	0.534	0.438	0.02	0.09	0.400	0.252
Osmolality, mOsm/kg	312.4	300.9	311.3	301.1	2.2	0.22	<0.0001	0.861
Rumen pH								
Average	5.93	5.94	6.26	6.01	0.04	0.37	0.001	0.157
Time < 5.6, min/d	79.72	78.97	45.41	111.65	8.63	0.24	<0.0001	0.088
Time < 5.2, min/d[6]	0.22	0.20	0.00	0.39	0.06	0.05	–	–
Area < pH 5.6, pH × min/d	0.287[ab]	0.307[ab]	0.124[b]	0.510[a]	0.05	0.004	0.016	0.135
Area < pH 5.2, pH × min/d[6]	0.06	0.03	0.00	0.11	0.02	0.02	–	–

[a,b]Means within a row with different superscripts differ by Tukey test ($P < 0.05$).

[1]7.75:92.25 forage:concentrate ratio.

[2]CTR = control (no feed additives); FO = functional oils (a blend of castor oil acid and cashew nut shell liquid fed at 400 mg/kg); M30 = monensin included at 30 mg/kg; M40 = monensin included at 40 mg/kg.

[3]Trt = treatment effect; Hr = hour effect.

[4]Hour represents the time of rumen fluid sampling at 6, 12, 24, 30, 36, 48, 54, and 60 h after abrupt transition.

[5]A:P = acetate:propionate ratio.

[6]Means were compared by nonparametric test (Kruskal–Wallis).

First 60 h after Abrupt Transition to High-Concentrate Diets

The first phase of abrupt transition from a high-forage diet to a high-concentrate diet was evaluated throughout 60 h. There was an interaction between treatment and hour after the abrupt transition for ruminal propionate proportion ($P = 0.028$). From 36 h up to 60 h, the ruminal propionate proportion for the M30 and M40 treatments was greater compared with that for the CTR treatment (Fig. 1a). However, feed additives had no effect ($P = 0.633$) on acetate molar proportion (Table 3). Therefore, the acetate:propionate ratio (**A:P**) ratio decreased ($P = 0.020$) when monensin was fed in both concentrations compared with when the CTR treatment was fed, without being different for the FO treatment. The M40 treatment had less ruminal butyrate molar proportion ($P = 0.039$) than the CTR treatment. However, the FO and M30 treatments did not differ from the others. Functional oils decreased the ruminal isovalerate proportion ($P = 0.020$) compared with the M30 treatment. Total VFA ($P = 0.257$), isobutyrate ($P = 0.730$), valerate ($P = 0.616$), ammonia ($P = 0.908$), and lactate concentration ($P = 0.088$) and osmolality ($P = 0.220$) were not different among treatments. Feed additives did not change the mean rumen pH ($P = 0.370$) or the time the rumen pH remained below 5.6 ($P = 0.242$) during the first 60 h after the abrupt transition. However, the M40 treatment had greater ($P = 0.004$) area under the curve pH 5.6 than the M30 treatment. All the treatments, except M30, had pH below 5.2 and, consequently, area under pH 5.2 throughout 60 h after the abrupt transition, leading to differences ($P = 0.047$ and $P = 0.022$, respectively).

Dry Matter Intake

Steers submitted to an abrupt transition from high-forage to high-concentrate diets had an average DMI of 9.45, 9.35, 8.68, and 8.54 kg/d for the CTR, FO, M30, and M40 treatments, respectively. There was interaction between treatment and time for DMI ($P = 0.008$; Table 4). Monensin treatments (M30 and M40) showed less DMI on d 3, 8, and 9 after challenge compared with the CTR diet ($P < 0.05$). The FO treatment had the greatest DMI compared with the other treatments on d 12, whereas on d 4, the FO treatment produced greater DMI than the M40 treatment and, on d 9, greater DMI than the M30 and M40 treatments, but on d 17, the least DMI was observed for steers consuming the FO diet compared with steers consuming the CTR diet ($P = 0.045$; Fig. 1b).

Table 4. Dry matter intake and rumen fluid fermentation patterns of Nellore steers at 21 d after abrupt transition to a high-concentrate diet[1] with different feed additives

	Treatment[2]					P-value[3]		
Item	CTR	FO	M30	M40	SEM	Trt	D[4]	Trt × D
DMI								
kg/d	9.45	9.35	8.68	8.54	0.11	0.50	<0.0001	0.008
Variation, kg	0.33	0.49	0.32	0.22	0.08	0.38	<0.0001	0.33
Ruminal parameters								
Total VFA, m*M*	115.77[a]	111.55[ab]	117.36[a]	105.02[b]	1.55	0.02	<0.0001	0.49
Acetate, mol/100 mol	55.96	54.69	54.43	56.35	0.99	0.52	<0.0001	0.95
Propionate, mol/100 mol	24.22	25.39	28.46	27.19	1.12	0.12	<0.0001	0.03
Butyrate, mol/100 mol	14.64[a]	14.63[a]	12.35[ab]	11.89[b]	0.71	0.01	<0.0001	0.27
Isobutyrate, mol/100 mol	1.17	1.09	1.07	1.03	0.03	0.61	0.004	0.17
Valerate, mol/100 mol	1.40	1.60	1.37	1.12	0.05	0.03	0.61	0.03
Isovalerate, mol/100 mol	2.75	2.68	2.49	2.61	0.09	0.58	<0.0001	0.22
A:P[5] ratio	2.59[a]	2.46[ab]	2.12[b]	2.29[ab]	0.06	0.03	<0.0001	0.35
NH_3–N, mg/dL	28.46	27.35	28.33	28.67	0.69	0.86	<0.0001	0.11
Lactate, m*M*	0.77[a]	0.62[b]	0.65[ab]	0.54[b]	0.02	0.001	0.56	0.08
Osmolality, mOsm/kg	340.9[a]	335.2[ab]	333.7[ab]	317.1[b]	3.0	0.04	<0.0001	0.60
Rumen pH								
Average	5.83	5.80	5.77	5.74	0.02	0.87	<0.0001	0.04
Minimum	5.25	5.23	5.20	5.18	0.01	0.91	<0.0001	0.25
Maximum	6.51	6.50	6.49	6.48	0.02	0.99	<0.0001	0.06
Time spent below, h/d								
Time < pH 5.6	8.03	8.32	8.88	10.37	0.31	0.74	<0.0001	0.20
Time < pH 5.2	3.17	2.26	3.02	2.15	0.22	0.88	0.001	0.10
Area under pH curve, pH × h/d								
Area < pH 5.6	2.86	2.65	2.76	2.63	0.16	0.98	0.0009	0.07
Area < pH 5.2	0.77	0.66	0.53	0.39	0.08	0.88	0.19	0.89

[a,b]Means within a row with different superscripts differ by Tukey test ($P < 0.05$).

[1]7.75:92.25 forage:concentrate ratio.

[2]CTR = control (no feed additives); FO = functional oils (a blend of castor oil acid and cashew nut shell liquid fed at 400 mg/kg); M30 = monensin included at 30 mg/kg; M40 = monensin included at 40 mg/kg.

[3]Trt = treatment effect; D = day effect.

[4]Day represents the days of rumen fluid sampling (d 1, 2, 3, 5, 7, 10, 13, 17, and 21). Dry matter intake and rumen pH were measured daily (d 1 to 21).

[5]A:P = acetate:propionate ratio.

Ruminal Fermentation Parameters

There was an interaction between treatment and day for propionate ($P = 0.034$) and valerate ($P = 0.031$) molar proportions. The M30 and M40 treatments increased propionate on the third day, whereas on d 5, propionate for the M40 treatment was still greater than propionate for the CTR and FO treatments (Fig. 1c). The FO treatment showed a greater valerate molar proportion than the other treatments on d 5, which reached the highest level on d 17, and, on d 21, that molar proportion was greater than that of the M40 treatment (Fig. 1d). Acetate ($P = 0.520$), isobutyrate ($P = 0.615$), isovalerate ($P = 0.578$), and ammonia ($P = 0.861$) concentrations were not affect by treatments (Table 4). However, the total VFA concentration was ($P = 0.017$) greater for the M30 (117.36 m*M*) and CTR treatments (115.77 m*M*) compared with the M40 treatment (105.02 m*M*), without being different from the FO treatment (111.55 m*M*). The treatments also affected butyrate concentration ($P = 0.014$), which was lower for the M40 diet than for the CTR and FO diets, and the acetate:propionate ratio ($P = 0.030$), where that of the M30 diet was lower than that of the CTR diet (Table 4). The M40 treatment decreased (317.1 mOsm/L) ruminal osmolality ($P = 0.040$) compared with the CTR treatment (340.9 mOsm/L), with no differences when compared with the FO (335.2 mOsm/L) and M30 treatments (333.7 mOsm/L; Table 4). The ruminal lactate concentration was lower ($P = 0.001$) for the FO (0.62 m*M*) and M40 (0.54 m*M*) treatments compared with the CTR treatment (0.77 m*M*). There was interaction effect ($P = 0.040$) between treatment and day after the abrupt transition for average daily pH (Fig. 1e). Feeding the M30 diet produced greater average pH (6.17) on d 3 compared with feeding the CTR (5.64) and M40 diets (5.56), without being different from the FO diet (5.83). Thereafter, the pH of all treatments declined until d 10. The pH for the M40 treatment was lower than the that of the CTR treatment on d 14 (5.58 vs. 6.02; $P = 0.025$), 15 (5.59 vs. 6.01; $P = 0.025$), and 19 (5.67 vs.

Table 5. Head-down events of Nellore steers abruptly fed a high-concentrate diet with different feed additives[1]

	Treatment[2]					Days						*P*-value [3]		
Parameter	CTR	FO	M30	M40	SEM	1	2	3	12	20	SEM	Trt	D	Trt × D
Duration, min/d	150.6	159.1	141.6	159.5	3.9	175.3[a]	152.6[bc]	132.0[c]	155.8[ab]	147.7[bc]	8.5	0.78	<0.0001	0.15
Frequency, events/d	99.6	70.5	73.7	63.4	2.8	90.8[a]	85.9[ab]	72.9[bc]	68.8[c]	64.4[c]	5.7	0.06	<0.0001	0.13
Eating rate, g DM/min	66.03	61.77	57.51	54.92	2.2	51.6[ab]	61.5[ab]	45.6[b]	61.7[ab]	80.5[a]	4.3	0.88	0.02	0.82

[a–c]Means within a row with different superscripts differ by Tukey test ($P < 0.05$).

[1]7.75:92.25 forage:concentrate ratio.

[2]CTR = control (no feed additives); FO = functional oils (a blend of castor oil acid and cashew nut shell liquid fed at 400 mg/kg); M30 = monensin included at 30 mg/kg; M40 = monensin included at 40 mg/kg.

[3]Trt = treatment effect; D = day effect.

6.23; $P = 0.003$), whereas the CTR treatment had greater average pH than the M30 treatment on d 14 (6.02 vs. 5.62; $P = 0.038$), 19 (6.23 vs. 5.74; $P = 0.012$), and 21 (6.08 vs. 5.69; $P = 0.045$). Average daily pH was lower ($P = 0.026$) for the FO diet (5.58) compared with the CTR diet (6.01) on d 15 but increased on d 19, when it was greater than that of the M40 diet (6.10 vs. 5.67; $P = 0.029$). No significant effect on minimum ($P = 0.910$) and maximum ($P = 0.985$) rumen daily pH, which averaged 5.21 and 6.49, respectively, was observed (Table 4). Feed additives did not change areas below pH 5.6 (average of 2.72 pH × h/d; $P = 0.980$) and 5.2 (average of 0.58 pH × h/d; $P = 0.883$) as well as the time spent below pH 5.6 (average of 8.9 h/d; $P = 0.738$) and below pH 5.2 (average of 2.65 h/d; $P = 0.884$).

Animal Behavior

No interaction between treatment and day was observed for any feed behavior variable ($P > 0.05$). The head-down duration ($P = 0.775$) and frequency ($P = 0.062$) and eating rate ($P = 0.836$) were not affected by treatments and averaged 152.71 min/d, 76.81 events/d, and 60.05 g DM/min, respectively (Table 5). However, days after the abrupt change in diet decreased head-down duration (175.3 min/d on d 1 vs. 147.7 min/d on d 20; $P \leq 0.0001$) and frequency (90.87 events/d on d 1 vs. 65.45 events/d on d 20; $P \leq 0.0001$), whereas the eating rate was least on d 3 (51.6 g DM/min) compared with d 20, which had the greatest eating rate (80.53 g DM/min).

Blood Chemistry

There was no ($P > 0.05$) interaction between treatment and days on any blood variables after the abrupt transition of diet. Feed additives affected pCO_2 ($P = 0.038$), with greater pressure for the M30 diet compared with the CTR diet, whereas greater HCO_3^- ($P = 0.006$) and tCO_2 ($P = 0.003$) were observed for the FO and M30 diets compared with the CTR diet (Table 6). Greater BE concentration ($P = 0.026$) was observed in the FO diet compared with the CTR diet and was not different compared with the BE concentration in the M30 and M40 diets. Conversely, no treatment effects were observed for PCV (averaged 33.97%; $P = 0.259$), osmolality (averaged 285.8 mOsm/L; $P = 0.294$), pH (averaged 7.390; $P = 0.332$), lactate (averaged 0.547 mmol/L; $P = 0.700$), pO2 (averaged 4.32 kPa; $P = 0.089$), and sO_2 (averaged 55.28; $P = 0.115$). Blood parameters over 21 d decreased for PCV ($P < 0.0001$), pH ($P < 0.0001$), and lactate ($P = 0.002$). On d 3, BE, HCO_3^-, and tCO_2 showed the lowest concentration and recovered from d 5 to 21 after transition.

Relative Population of Rumen Bacteria and Protozoa Counts

Interaction between treatment and day was not different ($P > 0.05$) for any bacteria population (Table 7). *Fibrobacter succinogenes* ($P = 0.465$), *Streptococcus bovis* ($P = 0.781$), and *Megasphaera elsdenii* ($P = 0.972$) strains were not affected by treatments. *Fibrobacter succinogenes* ($P < 0.0001$) and *S. bovis* ($P < 0.0001$) decreased their population throughout days, whereas *M. elsdenii* ($P = 0.026$) increased its population (Fig. 2). There was significant ($P < 0.0001$) effect of feed additive for the number of all ciliated protozoa genera assessed (*Entodinium*, *Diplodinium*, *Epidinium*, *Isotricha*, and *Dasytricha* spp.) as well the total ciliated protozoa concentration between treatments (Table 7). Nonetheless, some protozoa genera (*Ostracodinium*, *Eudiplodinium*, and *Enoploplastron* spp.), were not detected in the samples from either monensin treatment. The most abundant genus for all treatments tested compared with the total protozoa was *Entodinium*: 81.1% for the CTR treatment, 75.2% for the FO treatment, 92.8% for the M30 treatment, and 93.8% for the M40 treatment. Independent of ciliated protozoa genera, the greatest ($P < 0.0001$) protozoa counts were observed for the CTR treatment, were intermediate for the FO treatment, and were the least for steers fed monensin in both concentrations. Except the genera *Enoploplastron,* no ciliated protozoa count showed a day effect ($P > 0.05$).

Table 6. Blood metabolites of Nellore steers abruptly fed a high-concentrate diet[1] with different feed additives

	Treatment[3]					Days						P-value[4]		
Parameter[2]	CTR	FO	M30	M40	SEM	2	3	5	10	21	SEM	Trt	D	Trt × D
PCV, %	32.1	32.7	35.8	35.1	0.4	37.3^{a}	35.7^{ab}	34.7^{b}	32.0^{c}	30.0^{d}	1.04	0.26	<0.0001	0.75
Osmolality, mOsm/L	286.3	285.3	286.9	284.6	0.4	285^{ab}	284.5^{b}	285.3^{ab}	286.1^{ab}	288.0^{a}	0.88	0.29	0.02	0.30
pH	7.381	7.404	7.389	7.386	0.003	7.418^{a}	7.378^{b}	7.386^{b}	7.377^{b}	7.391^{b}	0.005	0.33	<0.0001	0.35
Lactate, mmol/L	0.56	0.48	0.52	0.63	0.03	0.88^{a}	0.60^{b}	0.51^{bc}	0.40^{cd}	0.33^{d}	0.07	0.70	0.002	0.80
pCO_2, mmHg	43.9^{b}	45.6^{ab}	46.6^{a}	46.1^{ab}	0.29	45.1^{b}	44.8^{b}	45.1^{ab}	46.2^{a}	46.5^{a}	0.64	0.04	0.03	0.37
pO_2, mmHg	33.8	32.8	30.3	32.6	0.40	32.1	31.9	33.6	32.5	31.7	0.90	0.09	0.83	0.78
BE, mmol/L	0.93^{b}	3.80^{a}	3.13^{ab}	2.60^{ab}	0.25	4.5^{a}	1.16^{c}	2.16^{bc}	1.96^{bc}	3.29^{ab}	0.52	0.0026	<0.0001	0.35
HCO_3^-, mmol/L	25.7^{b}	28.3^{a}	27.8^{a}	27.2^{ab}	0.22	28.8^{a}	26.2^{c}	26.8^{bc}	26.7^{bc}	27.8^{ab}	0.16	0.006	<0.0001	0.35
tCO_2, mmol/L	26.9^{b}	29.7^{a}	29.3^{a}	28.6^{ab}	0.24	30.1^{a}	27.3^{c}	28.1^{bc}	28.4^{abc}	29.4^{ab}	0.5	0.003	<0.0001	0.50
sO_2, %	57.7	57.1	50.5	55.7	0.85	56.8	53.6	57.9	54.2	53.7	1.9	0.115	0.72	0.79

[a–d]Means within a row with different superscripts differ by Tukey test ($P \leq 0.05$).

[1]7.75:92.25 forage:concentrate ratio.

[2]PCV = packed cell volume; pCO_2 = partial pressure of carbon dioxide; pO_2 = partial pressure of oxygen; BE = base excess; tCO_2 = total carbon dioxide; sO_2 = oxygen saturation.

[3]CTR = control (no feed additives); FO = functional oils (a blend of castor oil acid and cashew nut shell liquid fed at 400 mg/kg); M30 = monensin included at 30 mg/kg; M40 = monensin included at 40 mg/kg.

[4]Trt = treatment effect; D = day effect.

DISCUSSION

Ruminal acidosis occurs when cattle consume fermentable carbohydrates in sufficient amounts to cause nonphysiological accumulation of acids in the rumen with a concurrent reduction in pH (Nagaraja and Titgemeyer, 2007). The ability of monensin to modulate feed intake and prevent acidosis disturbance has been well reported (Nagaraja and Lechtenberg, 2007). Feeding monensin (M30 and M40) depressed DMI by 9% compared with the CTR diet (without feed additives). In a meta-analysis study, cattle fed a high-concentrate diet had reduction of DMI by 3.1% with monensin compared with a control diet (Duffield et al., 2012). Differences of DMI across treatments over the experimental period were more pronounced in the first week after challenge. On d 3, there was a severe drop of DMI for cattle fed the M30 and M40 diets compared with cattle fed the FO or CTR diets ($P < 0.05$). The DMI of steers decreased 24.6, 31.6, 42.1, and 46.3% for the CTR, FO, M30, and M40 diets, respectively, on d 3 compared with d 2. A decreased DMI within the first days after feeding high-concentrate diets appears to be common in feedlot animals. These results agree with Bevans et al. (2005) and Burrin et al. (1988), who tested a rapid transition from forage to a high-concentrate diet. However, as shown in this study, after the first week, DMI constantly increased (Nocek et al., 2002).

Total VFA concentration is related to the dietary composition and amount of intake (Aschenbach et al., 2011). In the current trial, this variable was reduced by 9% for M40 supplementation compared with the CTR. The lower VFA concentration led to a decreased ruminal osmolality observed for the M40 treatment than for the CTR treatment. Burrin and Britton (1986) reported that monensin shifts the VFA profile, although this action may be dose dependent (Ellis et al., 2012). Functional oil had no effect on total VFA concentration, which is in accordance with Cardozo et al. (2006). The increase of molar proportion of propionate might explain the DMI depression on d 3 after the abrupt transition in all treatments. Ruminal propionate is reported to regulate DMI (Allen, 1997) and it was greater than 25 mol/100 mol for monensin treatments, whereas it ranged between 19 and 21 mol/100 mol and between 22.6 and 23 mol/100 mol for the CTR and FO treatments, respectively, at 30 and 60 h after challenge. González et al. (2012) summarized some hypotheses to explain the reduction of DMI during subacute ruminal acidosis (**SARA**) episodes. These authors reported that DMI reduction may depend on the extent and convergence of multiple factors (high concentration of fermentation products and osmolality, inflammatory [acute-phase] responses, and reduced rumen motility) and that ruminal pH per se is not responsible for the DMI reduction. On d 3, we observed greater rumen mean pH and less time and area under pH 5.6 for the M30 diet compared with the M40 diet, which were not different from the others. These results were unexpected and may indicate the multifactorial aspects involved with DMI reduction or a monensin dose-dependent effect that is still unknown.

Similar to these results, Devant et al. (2007) observed no effect on acetate and isovalerate concentrations as well as a lower A:P ratio when young bulls were fed monensin (32 mg/kg) or a blend of essential oils (2,800 mg/kg), but no effect was described for ruminal propionate concentration compared with a control diet. Cashew nut shell liquid, one of the FO components, has been

Table 7. Bacteria relative population and protozoa counts (× 10^4/mL) of Nellore steers abruptly fed a high-concentrate diet1 with different feed additives[1]

Item	Treatment[2]				SEM	*P*-value[3]		
	CTR	FO	M30	M40		Trt	D	Trt × D
Relative population[4]								
Fibrobacter succinogenes	1.0	1.3	0.7	1.3	0.22	0.47	<0.0001	0.80
Streptococcus bovis	1.0	0.8	1.2	1.1	0.04	0.78	<0.0001	0.99
Megasphaera elsdenii	1.0	1.0	0.9	0.8	0.29	0.97	0.03	0.67
Genera								
Entodinium	42.81[a]	26.55[b]	13.93[c]	13.18[c]	2.17	<0.0001	0.32	0.31
Diplodinium	2.93[a]	1.01[b]	0.44[c]	0.37[c]	0.19	<0.0001	0.29	0.22
Epidinium	1.48[a]	0.42 [b]	0.15[c]	0.13[c]	0.10	<0.0001	0.47	0.11
Isotricha	2.00[a]	0.77 [b]	0.23[c]	0.22[c]	0.13	<0.0001	0.67	0.93
Dasytricha	1.89 [a]	0.71[b]	0.21[c]	0.14[c]	0.13	<0.0001	0.93	0.74
Ostracodinium	0.83	0.21	ND[5]	ND	0.08	<0.0001	0.19	0.06
Eudiplodinium	0.54	0.16	ND	ND	0.05	0.0002	0.24	0.35
Enoploplastron	0.25	0.14	ND	ND	0.02	0.002	0.01	0.01
Total	52.74[a]	35.27[b]	15.00[c]	14.05[c]	3.15	<0.0001	0.28	0.24

[a–c]Means within a row with different superscripts differ by Tukey test ($P \leq 0.05$).

[1]7.75:92.25 forage:concentrate ratio.

[2]CTR = control (no feed additives); FO = functional oils (a blend of castor oil acid and cashew nut shell liquid fed at 400 mg/kg); M30 = monensin included at 30 mg/kg; M40 = monensin included at 40 mg/kg.

[3]T = treatment effect; D = day effect.

[4]Changes in ruminal population based on the population size of steers fed the CTR diet.

[5]ND = no protozoa count detectable.

reported to decrease acetate and butyrate and increase propionate concentration in vitro (Watanabe et al., 2010) and in vivo (Mitsumori et al., 2014). In contrast, supplementation of FO in the present study did not change the molar proportion of acetate, propionate, and butyrate compared with a diet without feed additives (CTR).

Fandiño et al. (2008) also observed that molar proportion of butyrate decreased in cattle consuming monensin (238 mg/d) but was unaffected by essential oil (anise, 500 mg/d) compared with a control (no feed additives). According to the authors, these results indicate that monensin and essential oils may have different actions on rumen fermentation. This reduction can be associated with the inhibition of the Gram-positive bacteria *Butyrivibrio fibrisolvens* by monensin. Butyrate is one of the main products of this species (Russell and Strobel, 1989). Essential oil supplementation improved the butyrate molar proportion compared with the control only when doses were less than 0.02% and greater than 0.05% and produced, on average, a 5.6% reduction from a control (Khiaosa-ard and Zebeli, 2013). These findings are in agreement with the results from the current trial, where the FO supplementation at 0.04% did not have a distinct effect compared with the CTR diet.

Ruminal lactate concentration was below 1 m*M* for most of the experimental period. Neither an effect of day nor an interaction between treatment and day was observed. Despite this, the CTR treatment showed some increase, mostly on d 5 and 10 (data not shown). A ruminal lactate peak (2.01 m*M*) was registered in 1 steer consuming the CTR diet on d 5 after the abrupt challenge, whereas the greatest lactate concentration for other steers was observed on d 5 (1.53 m*M*), 21 (1.50 m*M*), and 21 (1.68 m*M*) for the FO, M30, and M40 diets, respectively. Different results were found by Brown et al. (2000), who reported that steers induced to acute and subacute acidosis challenges reached total ruminal lactate concentrations of 48.1 m*M* and 17.5 m*M*, respectively. Therefore, subacute acidosis that occurred in the present trial originated mainly due to the VFA accumulation, which resulted in decreased pH (Coe et al., 1999; Bevans et al., 2005).

Treatments had different average daily pH patterns over the experimental period; however, no differences among them were found. A long-term trial (119 d) conducted by Towne et al. (1990) showed that the ruminal pH effect by monensin had limited persistence over time. In the same way, Burrin et al. (1988) demonstrated that monensin was able to reduce acidosis by maintaining the ruminal pH only initially after transition. The rumen pH average recorded by the indwelling probes in the current study was 5.78, and the pH ranged from 5.21 to 6.49 for the minimum and maximum rumen pH, respectively. The result from the present trial was similar to rumen pH averages of 5.64 and 5.93 found by Meyer et al. (2009) and Vakili et al. (2013), respectively, when feeding high-concentrate diets. Time below a pH threshold is an important tool in characterizing acidosis prevalence. Rumen

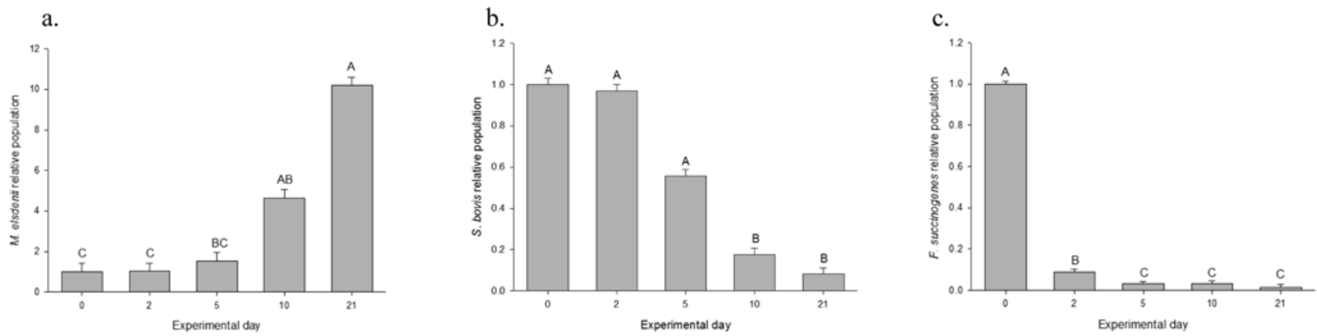

Figure 2. Bacteria relative population of *Megasphaera elsdenii* (a), *Streptococcus bovis* (b), and *Fibrobacter succinogenes* (c) of Nellore steers abruptly fed a high-concentrate diet with different feed additives. $^{A–C}$Means with different superscripts are statistically different by Tukey test ($P \leq 0.05$). Bars indicate SEM.

pH depression between 5.6 and 5.2, as well as below pH 5.2, for more than 12 and 6 h/d has been reported as subacute and acute ruminal acidosis, respectively (Owens et al., 1998; Bevans et al., 2005). However, over the 2 experimental periods (42 d) that rumen pH was monitored, all 6 animals from the CTR treatment showed more than 12 h below pH 5.6 at least 1 d, whereas the other treatments had 5 animals each with rumen pH below 5.6. On average, these animals presented 16.03, 16.40, 16.98, and 18.65 h/d below pH 5.6 for the M40, M30, FO, and CTR diets, respectively (data not shown).

Time spent below pH 5.6 and 5.2 were affected only by day after challenge. In this study, steers abruptly challenged with a high-concentrate diet had the greatest time spent below pH 5.6 on d 10 (13 h or 54.1% of a 24-h period), although the greatest time spent below pH 5.2 was observed later, on d 15 (4.74 h or 19.7% of a 24-h period).

The main objective of feed additive inclusion to a high-concentrate diet is to prevent ruminal disturbance by direct effects on ruminal fluid pH (changes on microbial population) or indirectly, by changes in feed behavior (González et al., 2012). In the present study, different feed additives did not modify the feeding intake pattern. One major advantage for supplementing monensin is its ability to modulate DMI, mostly by increasing the duration and frequency of DMI throughout days. These results agree with those of Erickson et al. (2003), who tested different monensin concentrations (0, 36.7, and 48.9 mg/kg), and Meyer et al. (2009), who compared the essential oil, monensin, and control diet (no feed additives) effects on feeding behavior. Both studies did not find an effect ($P \geq 0.05$) on time spent eating and number of meals per day. As far as we know, studies using FO have not reported an effect on feeding behavior. According to these results, this feed additive had no additional benefits on feeding behavior.

Blood pH was not different among treatments, but this variable decreased throughout days after the abrupt transition (pH 7.418 vs. pH 7.391 on d 2 and 21, respectively). However, blood pH remained within the physiological pH of 7.31 to 7.41 (Kaneko et al., 2008). Blood pH can be depressed by excessive acid production or insufficient acid removal (Owens et al., 1998). Hence, the lack of effect on blood lactate was expected.

Blood parameters during acidosis have been studied by many authors. Krehbiel et al. (1995) observed no effect on blood lactate, HCO_3^-, and pCO_2 in lambs experimentally induced to acidosis by increasing intraruminal infusion of glucose. Feeding steers the M30 diet had increased blood pCO_2 compared with feeding steers the CTR diet, without being different from steers fed the M40 and FO diets. Blood pCO_2 increased through d 10 and 21 compared with d 2 after the abrupt transition. An increase in blood pCO_2 has been observed in animals under SARA conditions (Morgante et al., 2009; Li et al., 2012).

According to Owens et al. (1998), high ruminal osmotic pressure pulls fluid from plasma into the rumen and consequently increases the PCV and osmolality of blood. However, in the present study, neither PCV nor blood osmolality were different among treatments.

Steers receiving the diet without feed additives (CTR) had less blood BE, HCO_3^-, and tCO_2 compared with steers receiving diets with feed additives. These blood variables are used as indicators of an animal's acid–base status (Radostits et al., 2007). These results agree with those of Brown et al. (2000), who found that steers experiencing acute acidosis had decreased blood HCO_3^-, BE, and tCO_2 as well as blood pH. Despite there being no treatment effect on blood pH and osmolality, the results might indicate an increased propensity for a systemic acid–base imbalance when the CTR diet was fed. Other trials have reported that a high-concentrate diet challenge affected the acid–base balance without affecting blood pH or PCV (Li et al., 2012). The use of different doses of monensin (0, 150, and 300 mg/animal) did not change blood pH or HCO_3^- (Burrin and Britton, 1986).

Fibrobacter succinogenes is a Gram-negative obligate anaerobic bacteria characterized as one of the major cellulolytic microorganisms in the rumen (Weimer, 1993). Conversely, *S. bovis* is the principal

species involved with rumen acidosis. This bacterial species exhibited an increased capacity to grow, especially when large amounts of starch are available, which lead to lactate production in the rumen (Russell and Hino, 1985). *Megasphaera elsdenii* is the most important lactate-using species in the rumen and also competes with lactate producers for glucose and maltose substrates (Henning et al., 2010). Neither monensin concentration nor FO affected the relative population of bacteria. In the present study, *F. succinogenes* showed a 64.9-fold decrease in relative population in steers fed a high-concentrate diet compared with steers fed a high-forage diet (hay during the baseline period) but a 5.73-fold decrease during the high-concentrate diet period (d 2 to 21). An abrupt transition in sheep also resulted in the decrease of cellulolytic bacteria (Grubb and Dehority, 1975). A beef cattle diet composed of a high concentrate led to a pH reduction and, therefore, less favorable conditions for fibrolytic bacterial populations (Petri et al., 2012).

A 11.9-fold decrease of *S. bovis* and a 10-fold increase of *M. elsdenii* in relative population were observed from d 2 to 21 of the high-concentrate feeding period. As the steers were adapted to the high-concentrate diet, an increase in DMI was observed and, consequently, an increase in the amount of starch ingested. *Streptococcus bovis* predominates under acidotic conditions, which reflects the tolerance of this species to proliferate even at low pH (Petri et al., 2013). Based on this, we expected that *S. bovis* would increase throughout days after the abrupt transition; however, the opposite occurred. The rumen lactate concentration in all treatments throughout days after transition remained below 3 m*M*, indicating that lactate did not accumulate. Therefore, the greatest time below pH 5.2 (acute acidosis threshold) registered on d 10 (227.8 min/d) after abrupt transition was due to increase in VFA concentration. Consequently, *S. bovis* had little influence on ruminal fermentation parameters, whereas the increase of *M. elsdenii* throughout the experimental period promoted a simultaneous competition for glucose and soluble sugar with *S. bovis* (Maroune and Bartos, 1987) and might explain the increase in propionate concentration. Also, there are other species such as *Selenomonas ruminantium* that were not measured in this assay and that produce propionate and have the ability to use a wide range of substrates (glucose, sucrose, and lactate; Russell and Baldwin, 1978; Fernando et al., 2010). Stepwise adaptation to high-concentrate diet resulted in 2-fold increase by the start of adaptation followed by a decreased by the end of step-up diet regimen (Fernando et al., 2010). The same authors also reported a gradual decrease of *F. succinogenes* populations and a fold increase in *M. elsdenii* as the animals were adapted to a high-concentrate diet, which is in agreement with the present study.

Considering the important role of ciliated protozoa in cattle fed high-grain diets (Nagaraja et al., 1992), by engulfing highly fermentable carbohydrates and fermenting them at a slower rate than bacteria (Mackie et al., 1978), the great protozoa count observed in the CTR diet may be beneficial for stabilizing the ruminal fermentation process. This may contribute to the higher pH during the second and third week after the abrupt transition (as shown in Fig. 1e).

In the present study, *Entodinium* spp. predominated in all treatments. According to Guan et al. (2006) this genera represented 91.1% of total protozoa when a high-concentrate diet (70%) with monensin (33 mg/kg) was fed to yearling steers. Khorrami et al. (2015) also reported that monensin or FO supplementation decreased the protozoa count in beef steers. Monensin administration, either M30 or M40, showed a more pronounced antiprotozoal effect than did supplementation of FO. However, on average, the FO treatment decreased total protozoa counts by 33% compared with the CTR treatment. Reductions of entodiniomorphids and isotrichids and concentration of total rumen ciliated protozoa were also found by Ando et al. (2003) and Fandiño et al. (2008) when peppermint and anise oil, respectively, were fed. However, according to Newbold et al. (2004), a blend of essential oils (thymol, guajacol, and limonene) had no effect on protozoal counts. The mechanism of action on protozoal count is still not clear but might be due tolipophilic nature of essential oils, which may permit them to cross through the protozoal membrane. Additionally, the dose–response effect of essential oils may explain the contradicting results among studies (Khiaosa-ard and Zebeli, 2013).

The present trial submitted Zebu cattle to an abrupt transition and observed that after a short-term adaptation period (first week), monensin or FO had little influence on ruminal parameters. Therefore, this study may contribute to new findings about the strategic use of ionophores and FO in finishing beef cattle diets.

There is not much information about abrupt transitions for *Bos indicus*, which has been suggested to be more susceptible to acidosis than *Bos taurus* (Millen et al., 2009); however, step-up protocols for Nellore cattle suggested 9 d for proper adaptation (Perdigão et al., 2017). Considering the results from the abrupt transition in this trial, further research is needed to elucidate *B. indicus* susceptibility to acidosis when abruptly feeding a high-grain diet.

Conclusion

Increasing monensin concentration (M40) and the use of FO did not change ruminal metabolism,

whereas 30 mg/kg of monensin decreased episodes of acidosis in feedlot animals, mainly by maintaining pH levels during the first week after the transition period.

This research clearly indicates the existence of SARA episodes, independent of which feed additive was used; therefore, the high-concentrate diet abruptly fed to unadapted Zebu cattle is still a practice that needs to continue to be investigated.

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