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## Effect of replacing antibiotics with functional oils following an abrupt transition to high-concentrate diets on performance and carcass traits of Nellore cattle

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### ABSTRACT

The aim of this study was to investigate the effects of different feed additives on performance, feeding behavior, blood metabolites, carcass traits and meat quality of finished Nellore cattle in feedlot, with abrupt transition to high-concentrate diets. Forty-eight 20-mo-old Nellore bulls [initial body weight (BW) of  $322 \pm 33$  kg] were distributed in a complete randomized design and fed with a basal diet containing different additives: 30 mg/kg dry matter (DM) of monensin (M30), 40 mg/kg DM of monensin (M40), 30 mg of monensin + 25 mg of Virginiamycin/kg DM (MV), and 400 mg/kg DM of Functional oils (FO; castor oil and cashew nut shell liquid). Animals were fed a diet of 92% concentrate on the first day of feedlot. Dry matter intake (DMI), body weight (BW) and blood parameters were evaluated at days 1, 7, 14 and 21 (transition period), then every 21 days to analyze performance and ultrasound carcass traits until slaughter. Animals fed FO had a greater DMI than MV ( $P = 0.002$ ) in the transition period, and showed no differences for M30 and M40, as well as for blood metabolites. In the feedlot period (120 days), the DMI was greater in FO than in M30 and in MV ( $P < 0.05$ ) with no differences from M40. The ADG, G:F and final BW, rumen parameters, carcass traits and meat quality showed no differences. However, the MV treatment had higher concentration of 15:0 and 17:0, 17:1, 18:1 t10, t11, t12 and 18:2, t10, c12 fatty acids. In conclusion, the abrupt transition of a diet to high concentrate did not affect performance and blood parameters. The FO did not affect negatively the performance, carcass traits and meat quality, compared to Nellore cattle in feedlot fed conventional additives.

**Abbreviations:** DMI, dry matter intake; ADG, average daily gain; BW, body weight; G:F, gain:feed; NE, net energy; FO, functional oil; CP, crude protein; TDN, total digestible nutrients; HCW, hot carcass weight; LMA, Longissimus muscle area; CL, coking loss; WBSF, Warner-Bratzler shear force; CLA, conjugated linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; UFA, unsaturated fatty acids; SFA, saturated fatty acids

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## 1. Introduction

Feed additives have been widely used in diets for beef cattle in feedlot to improve performance, profits and decrease production costs. Monensin and virginiamycin are ionophores widely used as growth promoters, due to positive effects such as reducing disease, increasing average daily gain (ADG) and improving gain:feed (G:F) and energy utilization (Tedeschi et al., 2003). However, there has been an increasing concern about the risk these products may present bacterial resistance and consequent possible risks to human health (Rivaroli et al., 2016).

In this sense, plant extracts and the functional oils (FO), oils that act over nutritional value, have been used as feed additives to replace conventional additives, and improve performance and meat production due to their antimicrobial, anti-inflammatory, antioxidant, and digestive modulatory effects on ruminal metabolism (Benchaar et al., 2006; Rivaroli et al., 2016).

The improvement in performance of cattle in feedlot is essential to provide economic success due to the high costs in feeding industry. Therefore, the inclusion of a fast diet is important to improve ADG and G:F (Zotti et al., 2017), specially for Brazilian feedlots that feed cattle for short periods (70–100 days; Millen et al., 2014). Zotti et al. (2017) evaluated the effects of different feed additives on ruminal fermentation and feeding behavior of cannulated *Bos indicus* (Nelore) cattle. According to the studies performed, a higher monensin concentration of 400 mg/kg DM and FO did not affect ruminal metabolism, whereas the monensin concentration of 300 mg/kg DM decreased acidosis in the transition period. Currently, no recordings on literature evaluated the effects of different feed additives (including FO) on performance, carcass traits and meat quality of *Bos indicus* cattle.

Therefore, we hypothesize that this FO blend could replace conventional antibiotics and decrease the risk of metabolic disorders in cattle fed high-concentrate diets preserving without negative effects on animal performance, carcass traits and meat quality. In addition, this study aimed to investigate the efficiency of these additives to avoiding ruminal and metabolic disorders, following an abrupt transition to a high-concentrate diet in feeding for *Bos indicus* (Nelore) cattle raised in pasture system with low performance and body weight, a common situation in Brazilian beef cattle production.

## 2. Material and methods

All procedures involving animals in this study were in accordance with the Institutional Animal Care and Use Committee Guidelines of the University of São Paulo and were approved by the animal ethics committee from the College of Animal Science and Food Engineering.

### 2.1. Experimental site and cattle

The study was conducted at the College of Animal Science and Food Engineering, from University of Sao Paulo, in Pirassununga/SP/Brazil. Forty-eight 20-mo-old Nelore bulls, with initial body weight (BW) of  $322 \pm 33$  kg were housed in individual concrete pens (2.0 m wide by 4.0 m deep; 2.0 m of linear bunk space) with access to feed and water ad libitum. Each treatment consisted of 12 animals randomly assigned and allocated in individual pens. The animals were kept in the feedlot for a total period of 120 days with an abrupt diet transition from 0 to 92% concentrate in the total mixture.

### 2.2. Abrupt diet transition, management and feeding

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. Twice daily, at 08:00 h and 16:00 h, the animals were fed a ground corn-based diet with an 8:92 forage (sugarcane bagasse):concentrate ratio on a dry matter (DM) basis (Table 1); the RLM software 3.2 calculated the experimental diets (Lanna et al., 2014). The amount of feed offered was adjusted daily according to the weight of the animals, which were maintained at approximately 5% of the feed offered. The treatments consisted of a basal diet with additives: 30 mg/kg DM of monensin (simulating the practical feedlot conditions (M30), 40 mg/kg DM of monensin (M40), 30 mg of monensin + 25 mg of virginiamycin/kg DM (MV), and 400 mg/kg DM of FO (Oligo Basics Agroindustrial Ltda., Cascavel, Paraná, Brazil). The FO used was a blend of castor oil acid and cashew nut shell liquid composed of cardanol (200 g/kg), ricinoleic acid (90 g/kg), and cardol (40 g/kg). The adopted FO dose was based on previous results of Zotti et al. (2017).

The variation in DMI was measured by the difference of consumption for two days as described by Schwartzkopf-Genswein et al. (2011) to evaluate the negative intake. The days with lower consumption concerning previous day were considered as negative consumption for the analysis.

### 2.3. Feed sampling and analytical procedure

Feed samples were collected every 21 days, selected according to specific treatments and frozen for later analysis of crude protein (CP) according to AOAC (2000), starch and ether extract (EE) according to AOAC (2005; AOAC (2005; Methods 996.11 and 2003.06, respectively). Neutral and acid detergent fiber (NDF; ADF) were determined using the ANKOM<sup>200</sup> Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) and AOAC International procedures (Official Methods 2002.04, and 973.18; 2005), respectively. Diet balanced on total digestible nutrient (TDN) was estimated according to Weiss et al. (1992) equation. Net energy for maintenance (NEm) and gain (NEg) were based on animal performance and calculated according to Zinn and Shen (1998).

**Table 1**  
Composition and nutrient content (DM basis) of the basal diet.

Parameter	g/kg
<i>Ingredient</i>	
Ground corn	820
Sugar cane bagasse	77.1
Soybean meal	68.3
Urea	14.4
Limestone	8.00
Trace mineral mixture <sup>1</sup>	6.00
Potassium chloride	6.00
<i>Nutrients</i> <sup>2</sup>	
TDN <sup>3</sup> , g/kg of DM	854
NEm <sup>4</sup> , MJ/kg	8.82
NEg <sup>4</sup> , MJ/kg	6.04
Crude protein, g/kg of DM	162
Starch, g/kg of DM	545
Ether extract, g/kg of DM	39.4
NDF, g/kg of DM	174
ADF, g/kg of DM	95.2

<sup>1</sup> The trace mineral mixture contained (per kilogram) zinc, 300 mg; calcium, 33 g; selenium, 1 mg; phosphorus, 15 g; manganese, 200 mg; copper, 100 mg; magnesium, 30 g; copper, 100 mg; cobalt, 4 mg; iodine, 7 mg; selenium, 1 mg; sodium, 68 g; sulfur, 47 g; sodium, 68 mg; potassium, 40 g.

<sup>2</sup> TDN = total digestible nutrients; NEm and NEg = net energy for maintenance and for gain, respectively; NDF and ADF = neutral and acid detergent fiber, respectively.

<sup>3</sup> Estimated according to Weiss et al. (1992).

<sup>4</sup> Estimated according to Zinn and Shen (1998).

#### 2.4. Performance and ultrasonography

To measure performance, the animals were weighed at the beginning of the feedlot period (day 1) and at days 7, 14, 21 and then every 21 days during the experimental trial (120 days). Weighings were performed without fasting to decrease the risk of acidosis and to minimize intake fluctuation. The ADG was calculated by difference between the initial and final BW. The G:F was calculated from ADG and DMI.

The longissimus muscle area (LMA) and backfat thickness (BFT), between 12<sup>th</sup> and 13<sup>rd</sup> were evaluated by ultrasound (Hitachi Aloka SSD500, Wallingford, CT, USA) using a 178 mm, 3.5-MHz probe, at day 1 and before slaughter. The images were saved in a portable computer and thereafter interpreted using the Lince<sup>®</sup> software (M&S Consultoria Agropecuária, Pirassununga, SP, Brazil).

#### 2.5. Blood sampling and analysis

Blood samples were collected at 1, 7, 14 and 21 days, 5 h after the morning feeding by punching the vena jugularis, using a flask containing no anticoagulant (BD Vacutainer, São Paulo, SP, Brazil). Blood samples were analyzed for pH, the blood pressure of carbonic gas (pCO<sub>2</sub>), base excess (BE), and bicarbonate (HCO<sub>3</sub><sup>-</sup>) and lactate concentrations. In addition, immediately after sampling, a few drops of blood were placed into an i-STAT EC8 +<sup>®</sup> cartridge to read the blood gas from a portable clinical analyzer (i-STAT<sup>®</sup> Co., Abbott Laboratories, EUA).

#### 2.6. Ingestive behavior

The behavioral intake was performed on the 27th, 56th and 92nd days, by visual observation of individuals every five minutes for 24 h, beginning at 16:00 h in the afternoon and ending at 16:00 h the following day. The observed variables were the time spent ingesting, ruminating, and idling as well as other activities according to the methodology proposed by Maekawa et al. (2002). Additionally, the ingestion rate was measured as kg of DM/ingestion time, minutes/day, and min/kg of DM; the rumination rate was measured as min/kg of DM; and the chewing rate was expressed as min/day and min/kg of DM.

#### 2.7. Slaughter, rumen and carcass samples

On day 120, the animals were transferred to the experimental slaughterhouse in the University of Sao Paulo, about 200 m distant from the feedlot facilities and slaughtered according to the Sanitary and Industrial Inspection Regulation for Products of Animal

Origin (Brasil, 1997). After evisceration, ruminal fluid sampling were taken to measure rumen pH. The rumen was examined, and the incidence of rumenitis was recorded according to Bigham and McManus (1975). The result was based on a scale from 0 to 10 points, and each point corresponding to 10% of compromised rumen. A sample of ruminal wall (1 cm<sup>2</sup>) was collected and prepared according to Resende-Junior et al. (2006) for morphological rumen evaluation.

The frequency of abscesses in the liver was evaluated according to the methodology described by Brink et al. (1990). Thus, a score of 0 for livers with no abscesses, 1 for one or two small abscesses (smaller than 2.5 cm) or abscess scars, 2 for two or four abscesses (greater than 2.5 cm), and 3 for more than four abscesses larger than 2.5 cm.

Hot carcass weight (HCW), initial carcass pH, temperature and the amount of internal fat (perirenal and inguinal fat depots) were recorded at slaughter. After 24 h of chilling, the final pH and temperature were recorded, and the longissimus muscle sample (2.5 cm thick) was taken from the 12th rib to measure its color, cooking loss, tenderness and fatty acid muscle profile.

## 2.8. Meat quality analysis

Instrumental color was analyzed using the CIELAB system (L\*a\*b\*), with a CR 200b Minolta colorimeter (Minolta Camera Co., Ltd, Osaka, OSA, Japan), with illuminant D65, a 30 mm aperture and a 10° observer angle. Three scans were taken of each sample and averaged to determine the instrumental color values. The Warner-Bratzler shear force (WBSF) and cooking loss (CL), were evaluated in *longissimus* samples aged for 8 days (0–2 °C) according the methodology described by the AMSA American Meat Science Association (AMSA, 1995) methodology. The steaks were thawed for 24 h at 4 °C, weighed, and roasted in an oven at 170 °C (Flexa de Ouro Industry, São Paulo, SP, Brazil). The internal temperatures of the steaks were monitored using individual thermometers until they reached 71 °C, and the steaks were then cooled at room temperature (24 °C) and weighed to measure the CL values. After that steaks were cooled at (4 °C) for 24 h, and then six cores were taken from each steak, parallel to the orientation of the muscle fibers, and sheared perpendicular to the muscle fiber using a WBSF instrument (Warner-Bratzler Meat Shear, G-R Manufacturing, Collins, KS, USA). The average of six cores was considered as the WBSF value.

To determine the fatty acid profile, muscle samples were saponified, and the fatty acids were extracted and methylated using the method of Hara and Radin (1978) and Christie (1982). The fatty acid profile was analyzed by gas chromatography (ThermoFinnigan, Trace 2000) using an SP-2560 silica capillary column (100 m × 0.25 mm in diameter with 0.02 mm thickness, Supelco, Bellefonte, PA). One standard (CRM-164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to identify the fatty acids.

## 2.9. Statistical analyses

All statistical analyses were conducted using SAS software (SAS Institute Inc., Cary, NC). Data were analyzed as a completely randomly design, using the MIXED procedure of SAS, considering the treatments M30, M40, MV or FO for fixed effects, and animal\*pen\*treatment for random effect. The initial BW was used as covariant for final and twenty-one day of BW analysis.

The DMI, blood parameters in the transition period and ingestive behavior variables were analyzed according to the number of times of replicated measurements, considering the days of evaluation as the replicate unit. The covariance structure was analyzed and the lowest value of AICC (autoregressive) was selected. When a significant effect was observed, means of treatments were compared using the LSMEANS adjusted for the Tukey test. The feedlot pens were considered as the experimental units.

## 3. Results

### 3.1. Abrupt diet transition: Performance and blood traits

The initial BW was similar between treatments, indicating homogeneity at the initial allocation (Table 2). However, during the abrupt diet transition, the DMI was affected by treatment in kg

( $P = 0.002$ ) and in percentage of BW ( $P = 0.038$ ). Bulls fed FO had a greater DMI ( $P < 0.05$ ), measured in kg, than those fed MV, but both did not differ from that of bulls in the M30 and M40 treatments. Additionally, animals fed FO had a greater DMI in percentage of BW, compared to MV and M40 treatments ( $P < 0.05$ ), but with no differences for M30. The M30 treatment did not differ from MV and M40 treatments.

No negative effect was observed as negative intake and BW measured at 21 days, as well as for any blood parameters, indicating no metabolic disorders due to the abrupt transition (Table 2).

### 3.2. Total experimental period: Intake and performance

Animals fed FO had a greater DMI (kg and % of BW;  $P < 0.05$ ) than those fed MV and M30, but they did not differ from bulls fed M40 (Table 3) during experimental period. There was no effect for ADG, G:F and final BW according to treatments in the experimental period. The NE (MJ/kg) for maintenance (NEm) and gain (NEg) were higher in animals fed to MV diet compared to the others ( $P < 0.05$ ).

**Table 2**

Effects of feed additives on intake, performance and blood parameters of feedlot Nellore cattle during an abrupt transition to a high-concentrate diet.

Traits <sup>1</sup>	Diets <sup>2</sup>				SEM	P
	M30	M40	MV	FO		
DMI, kg	5.87 <sup>ab</sup>	5.83 <sup>ab</sup>	5.35 <sup>b</sup>	6.56 <sup>a</sup>	0.27	0.002
DMI, % BW	1.71 <sup>ab</sup>	1.67 <sup>b</sup>	1.57 <sup>b</sup>	1.86 <sup>a</sup>	0.72	0.038
Negative intake, days	8.08	7.41	7.00	6.83	0.39	0.12
<i>Body weight, kg</i>						
d 1	318	326	320	324	6.84	0.84
d 21	349.9	351.3	341.4	354.3	3.79	0.11
<i>Blood parameters</i>						
pH	7.38	7.38	7.38	7.39	0.01	0.97
pCO <sub>2</sub> , mm Hg	45.7	45.1	45.7	44.4	0.93	0.72
pO <sub>2</sub> , mm Hg	41.7	40.1	41.4	40.2	1.17	0.86
Base excess, mmol/L	2.06	1.58	1.83	1.56	0.80	0.76
HCO <sub>3</sub> <sup>-</sup> , mmol/L	26.6	25.8	26.3	26.0	0.69	0.88
CO <sub>2</sub> , mmol/L	27.8	27.1	27.4	27.2	0.78	0.99
Lactate, mmol/L	3.14	3.84	3.79	2.34	0.64	0.31

<sup>abc</sup>Means within a row without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> DMI = dry matter intake.

<sup>2</sup> Diets: M30 - inclusion of 30 mg monensin/kg DM; M40 - inclusion of 40 mg monensin/kg DM; MV - inclusion of 30 mg monensin + 25 mg virginiamycin/kg DM; and FO - 400 mg functional oil/kg DM (Oligo Basics Agroindustrial Ltda., Cascavel, Paraná, Brazil).

**Table 3**

Effects of feed additives on intake and performance during the experimental period.

Traits <sup>1</sup>	Diets <sup>2</sup>				SEM	P
	M30	M40	MV	FO		
DMI, kg	7.7 <sup>b</sup>	8.5 <sup>ab</sup>	7.8 <sup>b</sup>	9.1 <sup>a</sup>	0.32	0.014
DMI, % BW	1.9 <sup>b</sup>	2.0 <sup>ab</sup>	1.9 <sup>b</sup>	2.1 <sup>a</sup>	0.06	0.015
ADG, kg/day	1.4	1.6	1.6	1.6	0.07	0.12
Final BW, kg	491	508	515	519	9.75	0.18
G:F, kg gain/kg DMI	0.18	0.18	0.20	0.18	0.01	0.10
<i>Observed NE<sup>3</sup>, MJ/kg</i>						
Maintenance	8.51 <sup>b</sup>	8.32 <sup>b</sup>	9.08 <sup>a</sup>	8.21 <sup>b</sup>	0.21	0.03
Gain	5.74 <sup>b</sup>	5.58 <sup>b</sup>	6.25 <sup>a</sup>	5.49 <sup>b</sup>	0.19	0.03

<sup>ab</sup>Means within a row without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> DMI = dry matter intake; ADG = average daily gain; BW = body weight; G:F = gain:feed; NE = net energy.

<sup>2</sup> Diets: M30 - inclusion of 30 mg monensin/kg DM; M40 - inclusion of 40 mg monensin/kg DM; MV - inclusion of 30 mg monensin + 25 mg virginiamycin/kg DM; and FO - 400 mg functional oil/kg DM (Oligo Basics Agroindustrial Ltda., Cascavel, Paraná, Brazil).

<sup>3</sup> NE = Net energy: estimated according to Zinn and Shen (1998).

### 3.3. Feeding behavior

There was an effect of additive type on the ingestion rate as measured as kg of DM/ingestion time (Table 4;  $P = 0.015$ ). The M30 and MV treatments had lower scores compared to M40 ( $P < 0.05$ ), but the FO treatment was similar to the others. Rumination and chewing rates were not affected by treatments, as well as feeding standards.

### 3.4. Rumen and carcass traits

There was no effect of additives on rumen pH, the incidence of rumenitis, papillae number and absorption area (Table 5) nor hepatic abscesses. In the same way, additives had no effect on the most carcass traits, except for internal fat, where FO had higher values when compared to the M30 and MV ( $P < 0.05$ ), but did not differ from M40 (Table 6).

### 3.5. Meat quality and fatty acid profile

The additives had no effect on the meat color ( $L^*a^*b^*$ ), CL and WBSF (Table 6). The percentages of saturated (SFA), mono-unsaturated (MUFA), and polyunsaturated fatty acids (PUFA), the PUFA:SFA ratio, n-3 fatty acids, n-6 fatty acids, and the n-6:n-3 ratio were also not affected by the type of additive (Table 7). In addition, there was no difference in the composition of most fatty acids between treatments, except for pentadecanoic (15:0;  $P = 0.033$ ), heptadecanoic (17:0;  $P = 0.023$ ), heptadecenoic (17:1;

**Table 4**

Effects of feed additives on feeding behavior of feedlot Nellore cattle during the experimental period.

Traits	Diets <sup>1</sup>				SEM	P
	M30	M40	MV	FO		
<i>Ingestion rate</i>						
kg DM/ingestion time	3.33 <sup>b</sup>	4.21 <sup>a</sup>	3.16 <sup>b</sup>	3.74 <sup>ab</sup>	0.34	0.015
min/day	159	148	169	159	10.66	0.60
min/kg of DM	20.8	17.6	21.7	18.9	1.58	0.26
<i>Rumination rate</i>						
min/kg of DM	30.7	30.9	33.3	30.0	1.65	0.53
<i>Chew rate</i>						
min/day	400	410	430	422	15.2	0.51
min/kg of DM	51.5	48.5	55.0	48.9	2.52	0.25
<i>Feeding standards</i>						
First feeding, min	21.6	26.2	23.7	22.5	2.05	0.42
Numbers of feedings/day	17.0	15.9	18.3	16.2	0.71	0.098

ab Means within a row without a common superscript differ ( $P < 0.05$ ).<sup>1</sup> Diets: M30 - inclusion of 30 mg monensin/kg DM; M40 - inclusion of 40 mg monensin/kg DM; MV - inclusion of 30 mg monensin + 25 mg virginiamycin/kg DM; and FO - 400 mg functional oil/kg DM (Oligo Basics Agroindustrial Ltda., Cascavel, Paraná, Brazil).**Table 5**

Effects of feed additives on rumen parameters of Nellore cattle.

Traits	Diets <sup>1</sup>				SEM	P
	M30	M40	MV	FO		
pH	6.35	6.40	6.30	6.45	0.16	0.91
Rumenitis incidence	1.60	0.90	0.85	1.02	0.52	0.72
<i>Papillae</i>						
Papillae area, cm <sup>2</sup>	0.75	0.73	0.71	0.61	0.07	0.59
Absorption area	38.9	38.2	41.1	33.9	4.40	0.83
Papillae number, cm <sup>2</sup>	51.7	51.6	55.3	54.0	4.60	0.94
% Papillae area	97.1	97.2	97.1	97.0	2.50	0.36

<sup>1</sup> Diets: M30 - inclusion of 30 mg monensin/kg DM; M40 - inclusion of 40 mg monensin/kg DM; MV - inclusion of 30 mg monensin + 25 mg virginiamycin/kg DM; and FO - 400 mg functional oil/kg DM (Oligo Basics Agroindustrial Ltda., Cascavel, Paraná, Brazil).**Table 6**

Effects of feed additives on carcass traits and meat quality of Nellore cattle.

Traits <sup>1</sup>	Diets <sup>2</sup>				SEM	P
	M30	M40	MV	FO		
HCW, kg	281	293	284	298	5.74	0.17
Carcass yield, %	57.8	57.3	55.7	57.2	0.75	0.26
LMA, cm <sup>2</sup>	64.3	65.0	66.7	68.4	2.20	0.42
Backfat thickness, mm	4.30	4.10	3.70	4.70	0.06	0.56
Internal fat, kg	8.89 <sup>c</sup>	10.5 <sup>ab</sup>	9.44 <sup>bc</sup>	11.0 <sup>a</sup>	0.45	0.007
Initial pH	6.68	6.73	6.58	6.75	0.07	0.37
Final pH	5.79	5.84	5.79	5.93	0.08	0.62
Initial temperature, °C	38.0	37.8	38.9	38.3	0.33	0.12
Final temperature, °C	5.91	6.04	5.92	6.10	0.13	0.67
<i>Color</i>						
L*	30.4	30.6	31.8	30.0	1.50	0.92
a*	15.1	14.5	15.8	15.4	0.86	0.85
b*	12.4	12.8	13.9	13.4	0.82	0.97
CL, %	27.1	28.6	27.4	27.9	1.18	0.84
WBSF, kg	6.61	6.12	5.88	6.86	0.45	0.43

abc Means within a row without a common superscript differ significantly ( $P < 0.05$ ).<sup>1</sup> HCW = hot carcass weight; LMA = Longissimus muscle area; CL = coking loss; WBSF = Warner-Bratzler Shear Force.<sup>2</sup> Diets: M30 - inclusion of 30 mg monensin/kg DM; M40 - inclusion of 40 mg monensin/kg DM; MV - inclusion of 30 mg monensin + 25 mg virginiamycin/kg DM; and FO - 400 mg functional oil/kg DM (Oligo Basics Agroindustrial Ltda., Cascavel, Paraná, Brazil).

**Table 7**  
Effects of feed additives on the *Longissimus* fatty acid profile of Nelore cattle.

Traits <sup>1</sup> , %	Chain length	Diets <sup>2</sup>				SEM	P
		M30	M40	MV	FO		
Myristic	C 14:0	3.36	3.50	3.56	3.36	0.19	0.86
Pentadecanoic	C 15:0	0.44 <sup>b</sup>	0.43 <sup>b</sup>	0.54 <sup>a</sup>	0.40 <sup>b</sup>	0.03	0.033
Palmitic	C 16:0	24.4	23.1	25.3	25.0	1.02	0.41
Heptadecanoic	C 17:0	1.10 <sup>b</sup>	1.14 <sup>b</sup>	1.34 <sup>a</sup>	1.03 <sup>b</sup>	0.07	0.023
Stearic	C 18:0	13.5	14.0	12.6	13.4	0.78	0.67
Arachidonic	C 20:0	0.08	0.08	0.08	0.08	0.006	0.87
Docosanoic	C 22:0	0.37	0.34	0.33	0.30	0.004	0.71
Myrtiloleic	C 14:1	0.85	0.93	0.94	0.90	0.04	0.53
Palmitoleic	C 16:1	3.23	3.57	3.44	3.43	0.09	0.12
Heptadecanoic	C 17:1	0.88 <sup>ab</sup>	0.87 <sup>b</sup>	1.01 <sup>a</sup>	0.78 <sup>b</sup>	0.06	0.060
Oleic <i>cis</i> -9	C 18:1	36.8	38.0	36.6	38.7	0.99	0.42
Trans-11 Vaccenic	C 18:1	1.54	1.51	1.52	1.51	0.05	0.98
Octadecanoic	C 18:1	1.35 <sup>a</sup>	1.09 <sup>ab</sup>	1.35 <sup>a</sup>	0.73 <sup>b</sup>	0.14	0.011
t10, t11, t12							
CLA c9,t12	C 18:2	6.33	6.00	5.84	5.19	0.50	0.44
CLA c9, t11	C 18:2	0.24	0.27	0.27	0.28	0.02	0.53
CLA t10, c12	C 18:2	0.011 <sup>a</sup>	0.009 <sup>a</sup>	0.009 <sup>a</sup>	0.004 <sup>b</sup>	0.001	0.006
Linolenic	C 18:3	0.43	0.40	0.41	0.37	0.002	0.43
Eicosatrienoic	C 20:3	0.01	0.01	0.01	0.01	0.00	0.64
Arachidonic	C 20:4	1.78	1.56	1.53	1.42	0.19	0.60
EPA	C 20:5	0.36	0.31	0.33	0.28	0.04	0.49
DHA	C 22:6	0.15	0.13	0.16	0.13	0.002	0.82
Σ saturated	–	43.8	43.2	44.4	44.2	1.16	0.88
Σ unsaturated	–	56.2	56.7	55.5	55.7	1.16	0.88
Σ monounsaturated	–	45.8	47.1	46.0	47.2	1.07	0.73
Σ polyunsaturated	–	10.38	9.67	9.48	8.55	0.84	0.50
UFA/SFA	–	1.30	1.33	1.26	1.29	0.006	0.90
Omega-3	–	1.80	1.62	1.63	1.47	0.17	0.58
Omega-6	–	8.44	7.91	7.72	6.6	0.68	0.50
Omega-3/omega-6	–	4.78	5.02	4.86	4.78	0.17	0.712

<sup>ab</sup>Means within a row without a common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup> CLA = conjugated linoleic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; UFA/SFA = unsaturated/saturated fatty acids.

<sup>2</sup> Diets: M30 - inclusion of 30 mg monensin/kg DM; M40 - inclusion of 40 mg monensin/kg DM; MV - inclusion of 30 mg monensin + 25 mg virginiamycin/kg DM; and FO - 400 mg functional oil/kg DM (Oligo Basics Agroindustrial Ltda., Cascavel, Paraná, Brazil).

$P = 0.060$ ), octadecanoic (18:1, t10, t11, t12;  $P = 0.011$ ) and conjugated linoleic acids (CLA 18:2, t10, c12;  $P = 0.006$ ). The MV treatment had higher values for 15:0 and 17:0 compared to the other treatments ( $P < 0.05$ ), while M30, M40 and FO did not differ. The MV treatment also had a higher concentration for 17:1 than in FO and M40 ( $P < 0.05$ ), but they did not differ from M30. The concentration of t10, t11, t12-18:1 was lower in FO compared to MV and M30 ( $P < 0.05$ ), however they did not differ from M40. The FO treatment also had lower concentration of t10, c12-18:2, than all treatments ( $P < 0.05$ ), but MV, M30 and M40 did not differ.

#### 4. Discussion

Over the past few decades, pharmaceutical technologies, such as antibiotics, have been used in beef cattle production systems to improve cattle performance and reduce production costs. Antibiotics are still the primary feed additive used in Brazil finishing diets, as previously reported by Oliveira and Millen (2014), who found that 98.7% of feedlots used some type of feed additive, highlighting ionophores as being predominant in these production systems.

The most consistent effect of antibiotics, such as monensin and virginiamycin, is the reduction of the incidence of metabolic disorders in cattle due to the finishing diet by controlling the pH in the rumen (Nuñez et al., 2013; Zotti et al., 2017). In a feedlot system, the rapid introduction of a finishing diet to beef cattle is necessary to improve ADG and the G:F ratio (Zotti et al., 2017), and this is extremely important in the Brazilian system since cattle remain on grass after weaning and must be adapted to high-concentrate diets at the beginning of the feedlot period. However, this adaptation period may decrease the compensatory gain, thus to maximize the gain, the abrupt change to a high-concentrate diet should be short, although this may increase the risk of metabolic problems. In this context, it is economically interesting to maintain greater DMI at the beginning of the feedlot period to maximize the compensatory growth and increase the carcass weight, but little is known about the effect of the abrupt high-concentrate feed system on Zebu beef cattle.

In the present study, increasing the energy density of the diet and the abrupt transition (0–92% concentrate) led to a drop in DMI (less than 2% BW) and ADG, probably due to the high intake of fermentable carbohydrates that results in a build-up of organic acids in the rumen, causing depression of the rumen pH and the consequent development of subacute ruminal acidosis (SARA) (Owens



et al., 1998). However, during the abrupt transition, animals fed FO had a greater DMI (measurement by kg/day and % of BW) than those fed MV, showing that the use of FO can promote a softer transition between diets at the beginning of the feedlot period. Zotti et al. (2017) also reported greater DMI for Nellore cattle fed FO compared to those fed M30 and M40 during the abrupt transition.

Furthermore, throughout the experimental period, the FO treatment increased the DMI by 14% and 13% compared to the M30 and MV treatments, respectively, without negative effect in performance. The increase in the molar proportion of propionate is very clear in the literature and this may lead an increase of propionate absorption, resulting in DMI depression due to quimiostatic factors in monensin diets (Allen et al., 2009). In agreement, Zotti et al. (2017) also reported that rumen cannulated Nellore steers fed a diet with monensin had lower DMI resulting from the greater than 25 mol/100 mol molar propionate proportion, whereas the proportion under the FO diet ranged between 22.6 and 23 mol/100 mol.

Purevjav et al. (2013) supplemented Angus and Angus crossbred steers with cashew nut shell liquid and castor oil and found no change in DMI. However, the MON improved ADG and G:F when compared with FO at both low (250 mg FO/kg DMI) and high doses (500 mg FO/kg DMI). Moreover, even with a reduction in DMI with the MV diet, the expected net energy for maintenance and gain contents of feeding have been improved, which suggests a possible additive effect of virginiamycin and ionophores. Nuñez et al., (2013) used the compound with virginiamycin and salinomycin in finishing cattle diets and observed this supplementation improved net energy for maintenance in 8.17%, and for gain in 10.63%, compared to the control diet.

Blood pH and electrolyte balance analyses are relevant in cattle fed high-grain diets because these parameters may be used to indicate animal health dysfunctions, such as SARA, when the findings of physical examinations are vague (Owens et al., 1998). According Radostits et al. (2007), decreases in blood pH and base excess (BE) with the subsequent increase in blood pCO<sub>2</sub> and tCO<sub>2</sub> can indicate animals under SARA conditions.

However, the use of different additives in the finishing diet of bulls did not alter the blood electrolyte balance in the present study, and this is supported by the lack of differences in the blood concentrations of HCO<sub>3</sub> and PCO<sub>2</sub> or their ratio, which is a major mechanism for maintaining the blood pH balance (Kaneko et al., 2008). Furthermore, all the values were within the accepted normal range (Kaneko et al., 2008), indicating, together with the rumen parameters, that all the additives used in this trial prevented the occurrence of metabolic disorders after the abrupt diet change.

Changes in feeding behavior, such as meal size or frequency, might indirectly affect changes in the pH of the rumen fluid due to feed additives in the diet (González et al., 2012). Feeding different rumen additives did not alter the time spent eating (average of 159.22 min/day), but the steers receiving M40 had the greatest ingestion rate (kg DM/hour intake). Strangely, this response was not observed when the lower concentration of monensin was provided (treatment M30). Erickson et al. (2003) observed that increasing the monensin concentration (33 or 44 mg/kg DM) did not affect the time spent eating compared to the control, but it increased the frequency and reduced the size of meals, which was not consistent with our results. These differences might be related to the distinct methodology of continuously recording individuals used by Erickson et al. (2003), whereas visual observations were adopted in the present study.

A combination of feed additive has been used in feedlot diets to improve animal performance (Nuñez et al., 2013). In the present experiment, MV changed the feeding behavior by lowering intake through the time spent eating and tended ( $P = 0.089$ ) to increase the number of feedings per day, which is important when considering the occurrence of acidosis and the need to better synchronize the time between acid production and its elimination or neutralization (González et al., 2012). The same combination of MON (30 mg/kg) and VM (25 mg/kg) was also used during the finishing period by Rigueiro et al. (2017), who observed changes in feeding behavior and reported a greater DM consumption rate (20.1 min. kg of DM vs 26.9 min. kg of DM) with MV compared to MON alone. These different DM consumption results can probably be explained by the higher proportion of concentrate in the diet used in the present trial compared to that used by Rigueiro et al. (2017).

There are few studies in the literature about FO and their effects on the feeding behavior of feedlot steers. Toseti (2017) reported a greater DM consumption rate (26.91 min. kg DM vs 18.89 min. kg DM) when feeding finishing steers FO (500 mg/kg DM) instead of MON (30 mg/kg DM). Conversely, other results have not indicated direct effects on the intake patterns of steers (Meyer et al., 2009; Zotti et al., 2017). Moreover, the use of FO and their capacity to change feeding behavior must be investigated further in future trials.

The papillae number, area and absorption area are important for increasing the SFA absorptive capacity, thus preventing excessive accumulation in the rumen and reducing the incidence and severity of ruminal acidosis (Melo et al., 2013). The basal diet had a high proportion of grain (82% ground corn on a DM basis), which can lead to high lactate production, decreased rumen pH, inflammation or degenerative processes such as ruminal mucosa, rumenitis and damage to the number and absorption area of the papillae. Nevertheless, no rumen damage was observed, suggesting that all the additives prevented rumen disorders and kept the rumen pH above 6.2, allowing for greater fiber degradation into the rumen. Generally, antibiotics and FO can act in the rumen to decrease the abundance of gram-positive bacteria, preventing the higher production of lactate from starch fermentation and resulting in fewer acidosis episodes (Nagaraja and Lechtenberg, 2007). Similarly, Zotti et al. (2017) verified no differences in rumen pH when they compared the use of monensin and FO in the diets of steers.

There have been studies of the correlation of feeding FO and abrupt diet transition with carcass traits, meat quality and the meat fatty acid profile. In the present study, the carcass traits and meat quality were generally not affected by feed additives. The mean pH value (5.93) was slightly above normal meat (5.5–5.8), but this could be a result of pre-slaughter management, which can cause stress, either psychologically or physically, and thus affect meat quality. However, no differences were observed in meat color, and there was no occurrence of dark meat, which is classified as having an L\* value below 29.7 (Abularach et al., 1998).

Among the polyunsaturated fatty acids (PUFAs), n-6 fatty acid (linoleic) and n-3 fatty acid (α-linolenic) are considered the most important because they are not synthesized by the organism and are the principal precursors of CLA. Total saturated fatty acids, unsaturated fatty acids or the polyunsaturated/saturated fatty acid ratio in the muscle was not affected by the dietary treatments,



with a mean of 43.9% saturated fatty acids and 56.1% unsaturated fatty acids. Nanon et al. (2014) reported that the inclusion of additives (FO) in the diet can affect ruminal biohydrogenation (RBH), of which the main role is to protect the ruminal ecosystem and microorganisms from unsaturated FA toxicity. Furthermore, fatty acid metabolism in the rumen plays a key role in fatty acid composition of beef (Jenkins et al., 2008).

In the present study, supplementation with a blend of FO decreased CLA 18:2, t10, c12, and these results might suggest alternative pathways in the production of the CLA C18:2, t10, c12 during fatty acid biohydrogenation depending on the type of additive. Wallace et al. (2007) reported that the rumen biohydrogenation (RBH) of linoleic acid (18:2) to CLA 18:2 c9, t11 is the process under normal conditions, but when pH is low and/or the diet is rich in starch (54% in this study), the RBH of linoleic acid (18:2) shifts to the CLA C18:2, t10, c12 pathway. In addition, the different standard of rumen modulation by additives also alters the rate of trans-octadecanoic acid production (18:1, t10, t11, t12), with greater values with M30 and MV than FO. Ferlay et al. (2017) observed that the fat content of ruminal fungi is rich in 18:1 fatty acid, and these microorganisms are able to desaturate 18:0 to cis9-18:1 and form a conjugated fatty acid (cis9,trans11-CLA) when they are incubated in the presence of 18:2n-6 and 18:3n-3. In this sense, the FO can have a greater fungicidal effect into the rumen.

## 5. Conclusion

In conclusion, the abrupt transition to high concentrate diets did not affect performance and blood parameters. The FO did not cause negative effects on performance, carcass traits and meat quality, compared to conventional additives for finishing Nelore cattle in feedlot.

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## Conflict of interest

There is no conflict of interest for none of the authors.

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