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Fatty acid profile and carcass traits of feedlot Nellore cattle fed crude glycerin and virginiamycin

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ABSTRACT

Forty-eight bulls with an initial body weight (BW) of 408.4 ± 22.2 kg (age = 21 ± 2 months) were used in a randomized complete block design arranged in a 2 × 2 factorial design. The treatments were diets without virginiamycin (VM−) or with virginiamycin, at 25 mg/kg of dry matter (DM, VM+), and diets without crude glycerin (CG−) or with crude glycerin, at 100 g/kg DM (CG+). The cold carcass weight and cold carcass dressing ($P \leq 0.05$) was greater in bulls fed crude glycerin diets. Total unsaturated fatty acids (UFA) were 6.08% higher, and cooking weight loss was 10% lower in bulls fed CG+ diets, compared to bulls fed CG− diets ($P = 0.0081$). Crude glycerin at 100 g/kg DM could be a suitable replacement for VM, as it led to a slight increase in UFA deposition in meat. However, simultaneous administration of VM and CG did not positively affect performance and carcass traits of feedlot Nellore cattle.

1. Introduction

Brazilian beef production from feedlots has increased in recent years, and market demands regarding efficiency and meat quality have also increased. Consumers are increasingly concerned and critical about the health characteristics of meat that can arise from intensive production practices, including those with a natural origin (fat content), or those associated with external compounds added to animals diets, such as antibiotics (T. McAllister & Cameron, 2016). On the other hand, modifying meat composition by feeding animals with byproducts or substrates that modulate fermentation may be a method to improve meat quality, and to reduce environmental impacts (Monteschio et al., 2017).

Feedlot diets are generally rich in concentrates. The faster rate of starch degradation from these diets increases the risk of metabolic disorders (e.g. acidosis). Several strategies have been used to prevent acidosis and increase beef cattle performance, including adaptation to grain diets (Bevans, Beauchemin, Schwartzkopf-Genswein, McKinnon, & McAllister, 2005), use of ruminal buffers (Crawford et al., 2008), use of antibiotics such as ionophores or virginiamycin (Coe et al., 1999), and administration of microbes such as *Megasphaera elsdenii* or *Sacchararomyces cerevisiae* (Meissner et al., 2010).

Antibiotics such as virginiamycin (VM) have been used in the feeding of livestock animals as a growth promoter. The VM inhibits the growth of gram-positive lactate-producing bacteria by disrupting protein synthesis (Cocito, 1979). Rogers et al. (1995) demonstrated, in a series of dose–response trials (19 mg to 27 mg VM/kg DM) with steers and heifers, that VM enhanced the average daily gain (4.6%), and gain to feed ratio (3.6%). However, studies describing the effect of VM on meat traits are still limited in beef cattle (Lemos et al., 2016; Salinas-Chavira et al., 2009), and, to our knowledge, no previous study has described fatty acid profiles of meat from feedlot Nellore cattle supplemented with VM.

The utilization of microbes that are directly fed to livestock, such as *Megasphaera elsdenii*, could be an alternative for antibiotics such as VM, by reducing ruminal accumulation of lactate (Counotte, Prins, Janssen, & Debie, 1981). However, many of the candidate microbes are obligate anaerobes, limiting cell yield, and complicating their culture in commercial fermentation facilities (T. A. McAllister et al., 2011). Thus, the use of glycerin, a highly available substrate from the biodiesel industry, that promotes the growth of *Megasphaera elsdenii* and *Selenomonas ruminantium* (Stewart, Flint, & Bryant, 1997) in the rumen, could be used to increase the proportion of lactate utilizing bacteria, and improve animal performance.

Crude glycerin (CG) has been demonstrated to have varying results when administered in livestock diets. In feedlot diets of beef cattle,

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glycerin levels up to 100 g/kg DM have been used to replace cereal grains, with positive effects on performance of beef cattle (Lage et al., 2014; Mach, Bach, & Devant, 2009; Parsons, Shelor, & Drouillard, 2009). Glycerin has also been demonstrated to enhance unsaturated fatty acid concentration of meat (Carvalho et al., 2014; Eiras et al., 2014; Favaro et al., 2016), possibly due to ruminal lipolysis inhibition (Edwards et al., 2012; Krueger et al., 2010).

We hypothesized that CG could replace VM without causing detrimental effects on the performance of feedlot Nellore cattle and fatty acid profiles of meat, and that the combination of VM and CG could increase animal performance and improve carcass traits of feedlot Nellore cattle. The objective of this study was to evaluate the effect of VM (0 or 25 mg/kg of DM) combined with CG (0 or 100 g/kg of DM) on the performance, and carcass and meat quality of feedlot Nellore Cattle.

2. Material and methods

2.1. Animals and feed management

This study was conducted in accordance with the Brazilian College of Animal Experimentation (COBEA – Colégio Brasileiro de Experimentação Animal), and it was approved by the Ethics, Bioethics, and Animal Welfare Committee (CEBEA; protocol number 021119/11). The experiment was designed as a completely randomized block and forty-eight Nellore bulls (21 months of age) with initial body weights (BW) of 408.4 ± 22.2 kg were individually fed feedlot diets without virginiamycin (VM−) or with virginiamycin, at 25 mg/kg of dry matter (DM, VM+/ V-Max, Phibro Animal Health, Ridgefield Park, NJ), and diets without crude glycerin (CG−) or with crude glycerin at 100 g/kg DM (CG+).

Crude glycerin was acquired from a soybean oil based biodiesel production company ADM, Rondonópolis, Brazil (80.3% glycerin; 1.59% ether extract; 5.03% ash, and 12.0% water). Cattle were first given 21 days of adaptation to experimental procedures and diets, and the experimental period to evaluate cattle performance and meat traits lasted 81 days.

Cattle were fed two times daily, at 07:00 and 16:00, and feed refusals were recorded daily for each pen. Amounts of feed offered to animals were calculated according to previous dry matter intake (DMI) and adjustments were made when needed, so that refused feed did not exceed 10% of daily intake. Orts daily weights and samplings were performed for the diet quantities provided for each animal. The ingredient proportions and chemical compositions of the experimental diets are presented in Table 1.

2.2. Sampling and chemical analysis

Forage and concentrate samples and orts from each animal were first composited weekly, and then composited every month. Feed samples were frozen at −18 °C for later analysis of DM and ether extract (AOAC, 1990). Nitrogen (N) content was determined by combustion (Leco Instruments Inc.), and multiplied by 6.25 to obtain crude protein (CP) content. Neutral detergent fiber (NDF) analyses were performed without sodium sulfite and with alpha amylase, and the data were corrected for residual ash (Mertens, 2002).

2.3. Slaughter, carcass data, and sample collection

At the beginning of the trial, all the cattle were weighed after a 16-h solid fast before the first feeding in the morning. The average daily gain (ADG) was obtained by weighing the cattle at the beginning and the end of the experiment, always after a 16-h solid fast. After 81 days of feeding, all the cattle (497.3 ± 41.9 kg) were transported to a slaughterhouse (Minerva Foods, Barretos, Sao Paulo, Brazil) 90 km from the feedlot. Transport to the slaughterhouse took approximately 1.5 h. On arrival at the slaughterhouse, cattle were kept in resting pens and were humanely slaughtered under Brazilian federal inspection. To compensate for potential differences in final BW due to variation in digestive tract fill and other factors, such as mud or manure on the animal, carcass adjusted final BW was calculated by dividing hot carcass weight for each animal by the average dressing percentage across all treatments within each study. After slaughter, carcasses were weighed and then refrigerated at 4 °C for approximately 24 h. After the postmortem chill period, the cold carcass weight (CCW), ultimate carcass pH (pHu), 12th rib fat thickness (RFT), and 12th rib longissimus muscle area (LMA) were measured on the left side of each carcass.

The LMA were traced on transparencies and measured later with a planimeter, and RFT measurements were taken at 3/4 of the length, ventrally over the longissimus muscle (Greiner, Rouse, Wilson, Cundiff, & Wheeler, 2003). Cold carcass dressing percent (CCD) was calculated using CCW divided by final shrunk body weight (SBW) and then multiplying the result by 100. A boneless longissimus section (10 cm thick) was removed from the posterior end of the whole rib. Longissimus muscle samples were individually vacuum-packaged and held at −20 °C for two days. Thereafter, each frozen longissimus sample was standardized from the posterior end into one 2.54-cm-thick steak sample (AMSA, 1995) for Warner-Bratzler shear force measurement, and other analyses, as described later. All steaks were vacuum-packed (99% vacuum, with a 200 Selovac Sealer machine, Selovac, São Paulo, SP, Brazil), in polyamide/polyethylene pouches of 120 μm and 1 cm^3/m^2/24 h 0_2 permeability, 3 cm^3/m^2/24 h CO_2 permeability measured at 5 °C and 75% relative humidity; water vapor transmission rate (WVTR)

Table 1
Chemical composition of the treatments.

Item		Treatments[a]			
		CG−		CG+	
		VM−	VM+	VM−	VM+
Ingredients g/kg DM					
Sugarcane bagasse		200	200	200	200
Ground corn		625	625	528	528
Soybean meal		136	136	131	131
Crude glycerin		0.00	0.00	100	100
Urea		9.00	9.00	11.0	11.0
Mineral premix[b]		30.0	30.0	30.0	30.0
Virginiamycin (mg/kg of DM)		0.00	25.0	0.00	25.0
Chemical composition, g/kg DM		852	852	828	828
Crude protein		157	157	156	156
Neutral detergent fiber		308	308	296	296
Ether Extract		25.0	25.0	25.0	25.0
Ash		74.0	74.0	76.0	76.0
Starch		372	372	322	322
Non fiber carbohydrates		436	436	447	447
Fatty acid (g/100 g of total FA)[c]					
Caprylic	8:0	0.03	0.03	0.02	0.02
Capric	10:0	0.02	0.02	0.02	0.02
Myristic	14:0	0.05	0.05	0.04	0.04
Palmitic	16:0	17.1	17.08	13.2	13.2
Margaric	17:0	0.17	0.17	0.11	0.11
Stearic	18:0	3.46	3.46	2.80	2.80
Palmitoleic	16:1 *cis*-9	0.15	0.15	0.12	0.12
Oleic	18:1 *cis*-9	39.6	39.6	37.0	37.0
Linoleic	18:2 n-6	35.0	35.0	41.0	41.0
α-Linolenic	18:3 n-3	1.14	1.14	1.51	1.51
SFA		20.96	20.96	16.3	16.3
UFA		75.74	75.74	79.6	79.6
MUFA		39.75	39.75	37.2	37.2
PUFA		36.14	36.14	42.5	42.5

[a] Diet without crude glycerin (CG−) or CG at 100 g/kg DM (CG+); diet without VM (VM−) or VM at 25 mg/kg DM (VM+).
[b] Composition (mg/kg of DM) = Calcium:8000; Phosphoros:3300; sodium: 2700; magnesium: 1400; sulphur: 6300; zinc: 82; cupper: 21; manganese: 52; cobalt: 1.3; iodine: 1.1: selenium: 0.35.
[c] SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

was 3 g/m^2/24 h at 38 °C and 100% RH. The vacuum value 20 (50 Pa) was used to pack the steaks, and steaks were stored at −20 °C for 10 days until analysis.

For proximate analysis, the epimysium was removed from the samples prior to lyophilization for 36 h. The cooking weight loss (CKL) was determined using thawed samples, as the difference between the weight of a steak before and after cooking in an oven pre-heated to 175 °C.

2.4. Meat and subcutaneous fat color

The determination of meat and fat color was performed as described by Cañeque et al. (2004), using a Minolta colorimeter (Model CR 400, Minolta Camera Co. Ltd., Osaka, Japan), and lightness (L*), redness (a*), and yellowness (b*) were evaluated. The color aspects were assessed by the CIE L*a*b* color system, using 0°/45°. Thirty minutes prior to the assessment, samples were removed from vacuum packages, and surface samples were exposed to air for oxygenation of myoglobin. The same procedure was used for the fat color measurement. After this step, the color was measured at three different points, and average values were calculated. The colorimeter was calibrated before analyzing the samples against white and black standards.

2.5. Warner–Bratzler shear-force measurement and cooking weight loss

The Warner–Bratzler shear force (WBSF) steaks were thawed at 4 °C for 24 h and oven-broiled in an electric oven (Layr, Luxo Inox) pre-heated to 150 °C. Internal steak temperatures were monitored by 20-gauge copper-constantan thermocouples (Omega Engineering, Stamford, CT) placed in the approximate geometric center of each steak, and attached to a digital monitor. When the internal steak temperature reached 35 °C, the steak was turned over and allowed to reach an internal temperature of 70 °C, before removal from the oven. Cooked WBSF steaks were cooled for 24 h at 4 °C (AMSA, 1995). Six round cores (1.27 cm diameter) were removed from each steak parallel to the long axis of the muscle fibers (Wheeler & Koohmaraie, 1999). Each core was sheared once through the center, perpendicular to the fiber direction, by a Warner-Bratzler shear machine (G-R Manufacturing Company, Manhattan, KS - USA). Cooking weight loss was evaluated for the steaks that were also used for WBSF measurement. Total cooking weight loss was calculated as the difference between the weight of the steaks before and after oven-broiling.

2.6. Fatty acid profile and fat content

For determination of the fatty acid composition of the fresh meat, samples of the transversal section were collected from the longissimus muscle, lyophilized, and frozen for lipid extraction and methylation. The fatty material was extracted using a mixture of chloroform–methanol, as reported by Bligh and Dyer (1959), and the fatty acid methyl esters (FAME) were obtained using the ISO 5509 method (1978). After phase separation, the upper layer was discarded, and 10 mL was transferred to glass beakers that had been previously tarred. After evaporation, beakers were re-weighed, and fat content was calculated based on the difference in weights. Qualitative and quantitative measurements of fatty acid content were performed by gas chromatography using a chromatograph (Model GC-14B, Shimadzu, Kyoto, Japan, with a Communication Bus Module - CBM 102) with a flame ionization detector (FID), and fused silica capillary column (Omegawax 250), which was 30 m in length and 0.25 mm in diameter, and had a film thickness of 0.25 μm (Supelco SP-24136). Helium was used as a carrier gas at a flow of 1 mL/min. A 1 μL aliquot of the sample was injected into a “split” at a division ratio of 1/100 and a temperature of 250 °C. The temperature of the oven was programmed to remain at 100 °C for 2 min, and then increase to 220 °C at 4 °C/min for 25 min, while the detector was set at 280 °C. Identification and quantification of the methyl esters of the fatty acids was achieved by comparison with the retention times and concentrations of methyl esters of standard fatty acids.

The indices of enzyme activities of Δ^9-desaturase for C18 fatty acids and C16 fatty acids and elongase were estimated according to Malau-Aduli, Siebert, Bottema, and Pitchford (1997):

$$\Delta^9 - \text{desaturase } 16 = 100[(\text{C16:1cis9})/(\text{C16: 1cis9} + \text{C16: 0})]$$

$$\Delta^9 - \text{desaturase } 18 = 100[(\text{C18:1cis9})/(\text{C18: 1cis9} + \text{C18: 0})]$$

$$\text{Elongase} = 100[(\text{C18: 0} + \text{C18:1cis9})/(\text{C16: 0} + \text{C16: 1cis9} + \text{C18: 0} + \text{C18:1cis9})]$$

The atherogenicity index and saturation index (SI) was estimated according to Ulbricht and Southgate (1991):

$$\text{Atherogenicity} = [\text{C12: 0} + 4(\text{C14: 0}) + \text{C16: 0}]/\Sigma\text{UFA}$$

$$\text{SI} = (\text{C14: 0} + \text{C16: 0} + \text{C18: 0})/\Sigma\text{MUFA} + \text{PUFA}$$

2.7. Statistical analysis

The data on animal performance and carcass variables were analyzed as a completely randomized block design, arranged in a 2 × 2 factorial design, including dietary virginiamycin and crude glycerin. The block was represented by two different feedlots, and the Nellore bulls were housed in individual pens (bull = experimental unit). Statistical models included virginiamycin (1 degree of freedom, DF), crude glycerin (1 DF), and all interactions as fixed effects. Block (1 DF) and residual error were included as random effects. Data were analyzed using the PROC MIXED procedure in SAS statistical software. When significant, the means between treatments were compared using the Fisher's least significant difference test (i.e., the option ‘pdiff command LSMEANS’). A probability of $P < 0.05$ was considered to be statistically significant, but tendencies with $P \leq 0.10$ were noted and discussed.

3. Results

There were no significant interactions between performance and carcass traits. Thus, the main effects of VM or CG on intake, average daily gain, and feed efficiency are reported independently (Table 2). The DMI, as a percentage of BW was greater in cattle fed CG+ (2.06%) diets, than in cattle fed CG− diets (1.95%), regardless of VM addition (Table 2; $P = 0.0351$). The ADG was 1.31 kg/day for cattle fed CG+ diets, and it was 1.13 kg/day for cattle fed CG− diets ($P = 0.0452$). The ADG was 1.30 kg/day for cattle fed VM+ diets, and 1.14 kg/day for cattle fed VM− diets ($P = 0.0832$). The NDF intake was statistically similar for all diets ($P = 0.1843$). Cattle fed diets with VM+ tended to have higher feed efficiency ($P = 0.0954$).

The final BW, RFT, LMA, and WBSF were similar among the treatments ($P \geq 0.1$; Table 3). Cattle fed CG+ diets had lower CKL and higher HCW, CCW, and CCD when compared with cattle fed CG− diets ($P < 0.1$). The fat content did not change in cattle fed diets with CG+ or VM+ ($P \geq 0.1$). Meat color, subcutaneous fat color, and pH did not change in cattle fed CG+ diets ($P \geq 0.1$; Table 4). However, the b* value of fat color tended to be lower in cattle fed VM+ diets ($P = 0.0901$).

The saturated and monounsaturated fatty acid profiles of longissimus muscles are reported in Table 5. There was an interaction effect between CG and VM for myristoleic acid ($P = 0.0815$). Myristoleic acid concentration had a tendency to be greater in longissimus muscles from cattle fed diets with VM (VM+) but without CG (CG−) than in longissimus muscles from cattle fed diets with CG+ combined with VM+ ($P = 0.0614$). The inclusion of dietary CG or VM did not affect the capric, lauric, myristic, and palmitic acid content of meat ($P \geq 0.1$). Heptadecanoic, margaric, and stearic acid concentration were greater in meat from cattle fed CG+ diets than in meat from cattle fed CG−

Table 2
Effect of virginiamycin (VM) and crude glycerin (CG) on intake, average daily gain (ADG) and feed efficiency (FE) of feedlot Nellore cattle.

Item	Treatments[a]							
	CG−		CG+			P-value		
	VM−	VM+	VM−	VM+	SEM[b]	CG	VM	CG × VM
Intake, kg								
Dry matter	9.00	10.1	10.2	10.4	0.41	0.0421	0.6112	0.4124
Organic matter	8.26	9.21	9.33	9.51	0.37	0.0443	0.2418	0.3884
Crude protein	1.29	1.39	1.48	1.52	0.07	0.0112	0.6635	0.3277
Neutral detergent fiber	2.46	2.73	2.69	2.80	0.13	0.1843	0.5843	0.1366
DM, % of BW	1.91	1.98	2.09	2.02	0.06	0.0351	0.9823	0.1525
ADG	1.04	1.22	1.23	1.38	0.08	0.0452	0.0832	0.7643
FE	0.11	0.12	0.11	0.13	0.01	0.2651	0.0954	0.3965

[a] Diet without crude glycerin (CG−) or CG at 100 g/kg DM (CG+); diet without VM (VM−) or VM at 25 mg/kg DM (VM+).
[b] Standard error of mean.

Table 3
Effect of virginiamycin (VM) and crude glycerin (CG) on carcass traits of feedlot Nellore cattle.

Item[b]	Treatments[a]							
	CG−		CG+			P-value		
	VM−	VM+	VM−	VM+	SEM[c]	CG	VM	GB × VM
No. of bulls	12	12	12	12	–	–	–	–
Initial BW, kg	410	419	414	412	14.6	0.8712	0.6745	0.4643
Final BW, kg	495	514	515	525	18.3	0.1634	0.2022	0.7363
HCW	282	287	298	304	9.05	0.0078	0.3232	0.8643
CCW, Kg	277	282	290	297	8.86	0.0328	0.3836	0.9073
CCD, %	56.9	55.7	58.2	58.3	0.91	0.0077	0.3557	0.4254
RTF, mm	4.1	5.58	4.66	4.79	0.54	0.8354	0.1582	0.2263
LMA, cm^2	69.9	71.2	74.0	74.9	3.05	0.1153	0.6522	0.9273
LMA/100 kg	25.2	25.3	25.6	25.2	0.70	0.8328	0.8645	0.7187
WBSF, kgf	4.85	4.32	4.69	4.78	0.40	0.6876	0.5564	0.3945
CKL, %	31.5	29.5	27.2	27.7	0.99	0.0081	0.4372	0.2148
Fat	2.29	2.34	2.46	2.59	0.23	0.4334	0.7354	0.8954

[a] Diet without crude glycerin (CG−) or CG at 100 g/kg DM (CG+); diet without VM (VM−) or VM at 25 mg/kg DM (VM+).
[b] HCW = hot carcass weight; CCW = cold carcass weight; CCD = cold carcass dressing; RFT = rib fat thickness; LMA = longissimus muscle área; LMA/100 kg = longissimus muscle area in relation to cold carcass weight; WBSF = Warner-Bratzler shear force; CKL = cooking loss.
[c] Standard error of mean.

Table 4
Effect of virginiamycin (VM) and crude glycerin (CG) on meat pH and subcutaneous fat color (L*, a* and b*)[b] of feedlot Nellore cattle.

Item[b]	Treatments[a]							
	CG−		CG+			P-value		
	VM−	VM+	VM−	VM+	SEM[c]	CG	VM	GB × VM
No. of bulls	12	12	12	12	–	–	–	–
pH	5.52	5.52	5.56	5.53	0.03	0.1867	0.4765	0.4432
Meat color								
L*	36.8	37	35.8	36.1	1.07	0.4233	0.8156	0.9822
a*	17.4	17.0	17.5	17.0	0.74	0.9465	0.4322	0.8776
b*	8.79	8.87	8.72	8.23	0.52	0.4934	0.6845	0.5765
Fat color								
L*	64.2	62.7	62.2	63.1	1.20	0.5033	0.8235	0.3445
a*	6.12	6.10	6.52	7.06	0.73	0.3455	0.7733	0.6733
b*	14.1	12.2	13.2	13.0	0.65	0.9567	0.0901	0.1934

[a] Diet without crude glycerin (CG−) or CG at 100 g/kg DM (CG+); diet without VM (VM−) or VM at 25 mg/kg DM (VM+).
[b] L* = luminosity; (0 = black and 100 = white); a* = index from green (−) to red (+); b* = index from blue (−) to yellow (+).
[c] Standard error mean.

diets, regardless of VM inclusion (P = 0.0254).

The polyunsaturated fatty acid profiles are shown in Table 6. There was a tendency for docosahexaenoic acid (DHA) to be greater in meat from cattle fed CG+ diets than in meat from cattle fed CG− diets (3.35 vs. 2.38 mg/100 g of meat; P = 0.0865). UFA (P = 0.0403) and monounsaturated fatty acids (MUFA) contents (P = 0.0391) were greater in meat from cattle fed CG+ diets than in meat from cattle fed CG− diets. The saturated fatty acid (SFA) content was 7.38% lower in meat from cattle fed CG+ diets than in meat from cattle fed CG− diets (P = 0.0396). The UFA/SFA (P = 0.0808) and MUFA/SFA ratio (P = 0.0320) was lower in meat from cattle fed CG+ diets than in meat from cattle fed CG− diets. However, the PUFA/SFA ratio was not affected by CG or VM (P ≥ 0.1). The n-6/n-3 ratio was similar in meat from cattle fed any of the diets (P ≥ 0.1). The Δ^9-desaturase enzyme activity index was greater for C18 fatty acids than for C16 fatty acids. Meat from cattle fed CG+ diets had a greater index of Δ^9-desaturase for C16 (P = 0.0725) and C18 (P = 0.0101) than meat from cattle fed diets without glycerin (CG−). The index of elongase and atherogenicity enzyme activity was not altered by VM or CG inclusion (P ≥ 0.1). The saturation index was lower in meat from cattle fed CG+ diets than in meat from cattle fed CG− diets (P = 0.0294).

4. Discussion

Feeding feedlot beef cattle diets containing CG at 10% DM did not affect their DMI (Lage et al., 2014; Mach et al., 2009; Schieck, Shurson, Kerr, & Johnston, 2010). In the present study, the increase in the intake of DM, OM, and CP in CG+ diets may be attributed to the fast rate of glycerin fermentation and epithelium absorption (Rémond, Souday, & Jouany, 1993). Another possible explanation for the increase in the DMI in cattle fed CG+ diets could be related to a reduction in non-esterified fatty acids (NEFA) concentration in the blood, as described in experiments with ewes during late gestation (Polizel et al., 2017), and primiparous dairy cows (Kass et al., 2012). The hepatic oxidation of fuels in liver (i.e., plasma NEFA) decreases the rate of hepatic vagal afferents, and results in satiety (Allen, Bradford, & Oba, 2009).

The increase in the DMI (7.25%) in cattle fed CG+ diets resulted in a greater CCW and ADG compared with cattle fed CG− diets. According to Waldo and Jorgensen (1981), DMI is a meaningful factor affecting animal performance, and a positive correlation between ADG and time spent ingesting feed can be expected (McGee et al., 2014).

The VM+ diets did not affect cattle DMI, but did tend to increase ADG and feed efficiency. Rogers et al. (1995) showed in a series of

Table 5
Effect of virginiamycin (VM) and crude glycerin (CG) on saturated and monounsaturated fatty acids (mg/100 g of meat) from longissimus muscle of feedlot Nellore cattle.

Fatty acid[b] (mg/100 g of meat)		Treatments[a]				P-value			
		CG −		CG +					
		VM −	VM +	VM −	VM +	SEM[c]	CG	VM	CG × VM
No. of bulls		12	12	12	12				
SFA									
Capric	10:0	1.54	1.24	1.55	1.41	0.26	0.7322	0.1945	0.6964
Lauric	12:0	1.83	1.62	1.78	1.64	0.17	0.9031	0.1444	0.9344
Myristic	14:0	82.4	76.0	76.6	76.7	6.37	0.9416	0.1854	0.9858
Palmitic	16:0	688	679	642	667	20.1	0.1734	0.9568	0.4768
Margaric	17:0	71.2	65.7	76.7	73.5	1.72	0.0077	0.2345	0.1076
Stearic	18:0	426	459	389	366	21.9	0.0082	0.8887	0.1254
MUFA									
Myristoleic	14:1 *cis*-9	17.2	15.4	17.3	20.4	1.87	0.2223	0.8535	0.0815
Palmitoleic	16:1 *cis*-9	77.9	73.7	77.1	85.9	4.35	0.1645	0.4764	0.4753
Heptadecenoic	17:1	18.7	18.1	24.8	31.4	1.37	0.0254	0.2355	0.1177
Oleic	18:1 *cis*-9	1057	1000	1084	1112	24.6	0.0210	0.8534	0.1898
Eicosenoic	20:1 *cis*-9	4.17	3.82	4.52	4.50	0.54	0.6133	0.4943	0.1423

[a] Diet without crude glycerin (CG −) or CG at 100 g/kg DM (CG +); diet without VM (VM −) or VM at 25 mg/kg DM (VM +).
[b] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids.
[c] Standard error of mean.

dose–response trials (19 mg to 27 mg VM/kg of DM) with steers and heifers, that VM enhanced average daily gain (4.6%), and gain to feed ratio (3.6%). These results demonstrated an increase in efficiency of energy utilization in cattle fed VM + diets compared with cattle fed VM − diets. Possible mechanisms involved in growth-performance enhancement include: inhibition of lactate producing bacteria (Coe et al., 1999; Nagaraja, Taylor, Harmon, & Boyer, 1987); increases in retention time and intestinal digestion (Ravindran, Kornegay, & Webb Jr, 1984) in pigs; and reduction in acetogenic bacteria content in the rumen, and facilitating growth of propionate-producing bacteria (Nagaraja et al., 1987; Van Nevel, Demeyer, & Henderickx, 1984), which are more energetically efficient.

Table 6
Effect of virginiamycin (VM) and crude glycerin (CG) on polyunsaturated fatty acids (mg/100 g of meat), Δ9-desaturase and elongase enzyme activity indices, and atherogenicity index from longissimus muscle of feedlot Nellore cattle.

Fatty acid (mg/100 g of meat)[b]		Treatments[a]				P-value			
		CG −		CG +					
		VM −	VM +	VM −	VM +	SEM[h]	CG	VM	VM × CG
No. of bulls		12	12	12	12				
PUFA									
Linoleic	18:2 n-6	131.3	140.6	161.5	141.6	15.8	0.3876	0.7167	0.3289
α-Linolenic	18:3 n-3	13.8	13.2	14.0	11.5	1.29	0.4644	0.1844	0.5348
CLA	18:2 *cis*-9. *trans*-11	12.1	10.5	11.1	11.9	0.93	0.8955	0.3613	0.1212
Eicosatrienoic	20:3 n-6	7.42	8.72	11.0	7.67	1.44	0.5967	0.1334	0.1388
Arachidonic	20:4 n-6	34.8	39.4	48.0	38.4	6.38	0.3248	0.5861	0.3835
EPA	20:5 n-3	10.2	10.9	12.8	10.0	1.92	0.3134	0.9827	0.3022
DTA	22:4 n-6	3.90	4.69	5.45	5.21	0.73	0.2788	0.8343	0.1734
DPA	22:5 n-3	18.7	19.7	24.8	19.6	3.13	0.4334	0.5712	0.2755
DHA	22:6 n-3	1.80	2.97	3.71	3.0	0.53	0.0865	0.8167	0.1465
Total SFA		1225	1242	1140	1145	42.13	0.0396	0.9514	0.7314
Total MUFA		1108	1179	1141	1180	30.60	0.0391	0.4555	0.2611
Total PUFA		204.4	220.6	256.5	218.6	25.5	0.5055	0.3842	0.2486
Total UFA		1312	1299	1397	1383	38.7	0.0403	0.9277	0.9454
PUFA/SFA		0.17	0.18	0.23	0.19	0.03	0.2738	0.5702	0.3132
MUFA/SFA		0.91	0.89	1.01	1.05	0.06	0.0320	0.9060	0.6410
UFA/SFA		1.08	1.02	1.13	1.16	0.05	0.0808	0.4845	0.7835
SI[c]		0.92	0.95	0.80	0.81	0.06	0.0294	0.6680	0.8490
n-6/n-3		4.08	4.10	4.00	3.98	0.19	0.9848	0.5855	0.9223
Δ^9-desaturase 16[d]		10.4	9.80	10.6	11.5	0.59	0.0725	0.7066	0.3168
Δ^9-desaturase 18[e]		70.9	69.2	72.8	75.3	1.37	0.0101	0.7843	0.1334
Elongase[f]		65.7	66.5	66.8	66.3	0.73	0.5824	0.8113	0.3531
Atherogenicity[g]		0.80	0.77	0.72	0.71	0.05	0.1743	0.7818	0.8152

[a] Diet without crude glycerin (CG −) or CG at 100 g/kg DM (CG +); diet without VM (VM −) or VM at 25 mg/kg DM (VM +).
[b] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids.
[c] SI = Saturation index.
[d] Δ9 desaturase 16 = 100[(C16:1cis9) / (C16:1cis9 + C16:0)].
[e] Δ9 desaturase 18 = 100[(C18:1cis9) / (C18:1cis9 + C18:0)].
[f] Elongase = 100[(C18:0 + C18:1cis9) / (C16:0 + C16:1cis9 + C18:0 + C18:1cis9)].
[g] Atherogenicity = [C12:0 + 4(C14:0) + C16:0] / ΣUFA.
[h] Standard error of mean.

The VM+ diets did not modify LMA/100 kg of cold carcasses, or RFT, which is probably due to an indirect lack of effects on adjusted final BW and HCW (Salinas-Chavira et al., 2009). The CG+ diets also did not affect LMA/100 kg of cold carcasses, which can be attributed to the genetic similarity between the cattle used in the experiment (Lage et al., 2014). The RFT (4.78 mm) did not differ significantly between cattle fed the different diets. The values of RFT (3 to 6 mm) met the requirements of the Brazilian beef industry. Other studies using CG in feedlot diets have reported similar RFT (Carvalho et al., 2014; Favaro et al., 2016; Ribeiro, Messana, Neto, Fiorentini, & Berchielli, 2016).

The use of dietary VM or CG did not affect WBSF, and the average value across our treatments (4.6) represents an overall consumer's satisfaction perception for tenderness (Platter et al., 2003). The slight decrease in CKL in cattle fed CG+ diets would not change consumers' acceptability of meat. This reduction in the CKL values may be explained by a high osmotic pressure in the muscle induced by glycerin, as demonstrated in pigs (Mourot, Aumaitre, Mounier, Peiniau, & François, 1994), and by the interaction of glycerin and protein during the cooking process (Lacroix & Castaigne, 1985). Although a large proportion of glycerin is fermented in the rumen (Claudia Kijora et al., 1998), some reports suggest that a portion may be absorbed in the epithelium, or flow to other parts of the body (Rémond et al., 1993), which increases its concentration in the bloodstream. This reduction in the CKL in cattle fed CG+ would not.

The mean carcass pH across the treatments with CG and VM was 5.53. Meat of high quality has a pH in the range of 5.4–5.6, and pH can affect meat quality traits, such as color (Weglarz, 2010). The diets used in the present study probably had the potential to maintain glycogen concentration at the time of slaughter, as insufficient glycogen at slaughter results in pH values above 5.5, which, in extreme cases, leads to a serious quality problem known as dark-cutting (Immonen, Ruusunen, Hissa, & Puolanne, 2000). Although diets containing glycerin would have the potential to increase glycogen concentration due to availability for ruminal and duodenal metabolism, CG+ diets did not change ultimate pH values of the carcasses.

Meat color is an indicator of freshness, and it represents the main quality factor affecting purchasing decisions of consumers (Mancini & Hunt, 2005). In the present study, meat from cattle fed CG+ and VM+ diets had mean values of 35.4, 17.2, and 8.25 for brightness, redness, and yellowness, respectively. The results of the present study are in accordance with Muchenje et al. (2009), who describe in a review of biochemical aspects of meat quality a range of values from 33.2 to 41, 11.1 to 23.6, and 6.1 to 11.3, for brightness, redness, and yellowness, respectively.

Favaro et al. (2016) have shown a reduction in b* value in meat due to the inclusion of glycerin at 10 and 15% of DM, replacing corn grains. The authors reported a possible reduction in carotenoid intake due to replacement of corn with CG during the formulation of diets. The lack of effect in the present study can be attributed to the fact that we used sugarcane bagasse as a source of forage. During the production of sugarcane bagasse by-products, the higher temperatures and grinding process reduces the concentration of carotenoids (Dunne, Monahan, O'Mara, & Moloney, 2009), and the combination of sugarcane bagasse with CG+ diets (e.g., low corn inclusion) did not compensate for the reduction in b* indices. Lage et al. (2014) evaluated meat quality of Nellore cattle fed diets with 10% crude glycerin replacing corn or soybean hulls, and also did not find any difference in beef color when glycerin was replaced by corn grain. The authors suggested that inclusion of crude glycerin at 10% DM was not sufficient to promote changes in meat and fat color.

Experiments evaluating carcass traits in feedlot diets with VM addition are scarce, and they did not demonstrate effects on fat color (Boucque, Fiems, Cottyn, & Buysse, 1990; Salinas-Chavira et al., 2009). However, in in the present study, VM+ diets decreased b* values of fat, which can be explained by the rate of gain. The VM+ diets tended to increase ADG compared with VM− diets. Knight, Death, Lambert, and McDougall (2001) conducted an experiment in which three groups of steers were fed with the objective of gaining weight rapidly, gaining weight slowly, or losing weight, with a fourth group of steers slaughtered at the outset. The yellowness and carotenoid concentrations of subcutaneous fat of the steers gaining weight rapidly were significantly lower than the group slaughtered at the outset, or those losing weight.

The nutritional value of beef has become increasingly important, as consumers are increasingly aware of the relationship between the consumption of certain fatty acids and cardiovascular health, which likely affects purchasing decisions (Buchanan et al., 2015). The CG+ diets increased MUFA and UFA content of meat, and decreased the proportion of SFA. Studies have suggested that glycerin may increase UFA absorption in the duodenum, due to a possible inhibition of ruminal lipolysis (Edwards et al., 2012; Krueger et al., 2010), and that glycerin may increase the duodenal flow of UFA (Granja-Salcedo et al., 2017), with enhancement of UFA deposition on meat (Carvalho et al., 2014; Eiras et al., 2014; Favaro et al., 2016).

Buchanan et al. (2015) evaluated genetic parameters for intramuscular fatty acids of Angus cattle, and found that the genes that cause increased levels of SFA do so at the expense of reduced levels of MUFA, or vice versa, which is in accordance with our results. These effects can be regulated by stearoyl-CoA desaturase (SCD), which introduces a double bond between carbon atoms 9 and 10 of a SFA to produce C14:1, C16:1, and C18:1 (Matsuhashi et al., 2011). Of the SFA, only margaric acid and stearic acid were lower in cattle fed CG+ diets in the present study. The reduction in stearic acid may be due to a reduction in the biohydrogenation of linoleic acid, which could limit the hydrogenation of vaccenic acid to stearic acid (Lage et al., 2014), a function of ruminal lipolysis inhibition (Edwards et al., 2012). On the other hand, the reduction in margaric acid concentration may be related to the low concentration in diets without CG. Both margaric and stearic acid have been demonstrated to have no net impact on serum cholesterol concentrations in humans (Daley, Abbott, Doyle, Nader, & Larson, 2010).

Heptadecenoic acid (C17:1) is synthesized by rumen bacteria from propionate (Berthelot, Bas, Schmidely, & Duvaux-Ponter, 2001), and its increase may be related to the ruminal fermentation of glycerin, which increases the proportions of propionate and butyrate at the expense of acetate (Bajramaj et al., 2017; Castagnino et al., 2015; C. Kijora et al., 1998). In our experiment, we quantified the rumen microbes (unpublished data), and found that the addition of glycerin increased the microbial N flow to the small intestine, which could explain the higher deposition of heptadecenoic acid in meat. The enhancement of heptadecenoic acids due to CG+ diets can provide healthy anti-carcinogenic effects for humans (Wongtangtintharn, Oku, Iwasaki, & Toda, 2004). Oleic acid deposition in the longissimus muscle was higher in cattle fed CG+ diets, probably due to the action of SCD, which corresponds with the higher index of Δ^9-desaturase enzyme activity for C18 fatty acids in CG+ diets. These results are in agreement with previous studies by (Lage et al., 2014), who observed an increase in the content of oleic acid in meat from feedlot Nellore cattle fed diets containing CG.

Overall, concentrations of UFA, and the UFA/SFA ratios were greater in meat from cattle fed CG+ diets, compared to meat from cattle fed VM+ diets. However, these differences were small, and are not considered sufficiently significant to have positive effects on human health. The PUFA/SFA ratio in meat remained similar among treatments (0.19), and was lower than the recommend value (0.4) for red meat in human diet (Department of Health, 1994).

Human intake of UFA has been demonstrated to reduce the risk of cardiovascular disease, and possibly the incidence of some cancers, asthma, and diabetes, among other conditions (Milicevic et al., 2014). Diets with VM+ had a tendency to slightly increase ADG and feed efficiency when compared with VM− diets. However, VM+ did not enhance the unsaturated fatty acid content in the meat.

5. Conclusions

Crude glycerin at 100 g/kg DM could be a suitable replacement for VM, as it led to an increase in UFA deposition in meat, although this increase was probably too small to have significant health benefits to human consumers of the meat. Simultaneous administration of VM and CG did not positively affect performance and carcass traits of feedlot Nellore cattle.

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