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Crude glycerin in corn grain-based diets for dairy calves

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ABSTRACT - The objective of this study was to evaluate performance, apparent digestibility, blood parameters, and quantitative characteristics of carcass and internal organs of crossbred dairy calves fed different levels of crude glycerin in corn grain-based diets. Twenty-four calves of three months of age and average initial weight of 95.5±11.8 kg were used. The experimental design was completely randomized with four treatments (0, 80, 160, and 240 g kg^{-1} of crude glycerin of DM of diets). The calves were fed in feedlot until six months of age (195.68±2.38 kg of BW). Dry matter (4.14, 4.11, 3.80, and 3.49 kg day^{-1}) and apparent digestible energy intake (0.43, 0.41, 0.37, and 0.35 MJ kg^{-1} BW) decreased with increasing levels of glycerin in the diets. There was no effect on the apparent digestibility of nutrients, average daily gain, feed efficiency, carcass characteristics, and blood parameters. The diets did not influence the weights [g kg^{-1} of empty body weight (EBW)] of lungs, heart, kidneys, liver, omasum, abomasum, and large intestine. The reticulorumen weight (g kg^{-1} EBW) increased, whereas the small intestine weight decreased with increased levels of glycerin in the diets. The area, height, and width of the rumen papillae were not changed with increasing levels of glycerin. The rumen wall thickness increased with increasing levels of glycerin in the diets. The inclusion up to 240 g kg^{-1} DM of crude glycerin of in corn grain-based diets for the production of dairy calves does not alter animal performance, carcass characteristics, and weights of internal organs.

Keywords: average daily gain, blood glucose, dry matter digestibility, empty body weight, hot carcass weight

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1. Introduction

The current Brazilian dairy production system, which discards male calves, needs to find more efficient and humane ways to raise these animals. Among the available technologies, the production of pink meat calves is highlighted, with solid diets (grain-fed veal), contrasting the milk-fed veal. Grain-fed veal is an interesting alternative, since it has similar characteristics to the meat from adult cattle and meets the demand for quality and health products for being considered lean and with low fat (Abu Bakar et al., 2001).

However, the rearing of cereal-fed dairy calves can have a relatively high production cost (Missio et al., 2009a). In this context, crude glycerin is considered as the new corn for dairy cattle (Donkin, 2008) and can help to reduce feed costs in feedlot (Saleem and Singer, 2018). The increase in the use of biodiesel in Brazil has led to a large production of glycerin, and because it has the largest commercial herd in the world, it shows great potential for the commercial absorption of this byproduct as an ingredient in ruminant diets (Barros et al., 2018).

Crude glycerin has been evaluated in cattle diets, and the results have indicated that there is an improvement in the average daily gain (ADG) and feed conversion (Moreira et al., 2016; Barros et al., 2018). Furthermore, the results of crude glycerin use seem to depend on diet composition (Hales et al., 2013), level of inclusion of this byproduct (Benedeti et al., 2016), and animal category (Maciel et al., 2016a; Maciel et al., 2016b).

We hypothesized that crude glycerin is potentially a large alternative source of energy available to replace corn in diet of the grain-fed veal. Therefore, we aimed to evaluate the nutrient intake and digestibility, animal performance, blood parameters, carcass characteristics, and internal organ characteristics of dairy calves fed varying levels of crude glycerin in corn-based diets.

2. Material and Methods

The experimental procedure was approved by the local Institutional Animal Care and Use Committee (case number 23101.003936/2012-00).

Twenty-four crossbred dairy calves (Holstein-Zebu) with an average initial weight of 95.5±11.8 kg at three months of age were allocated into the four diets using a completely randomized design. The calves were raised in a system of outdoor houses, receiving 4 L/day of milk and pelleted commercial (180 g kg^{-1} DM of crude protein of diets) *ad libitum* until two months of age. Between two and three months of age the calves remained in a pasture of Massai grass receiving ration based on corn grain and soybean meal *ad libitum* (160 g kg^{-1} DM of crude protein of diets). Thereafter, the calves were maintained in feedlot with individual covered pens with concrete floor, feeders, and drinkers.

The treatments were diets containing levels of crude glycerin (0, 80, 160, and 240 g kg^{-1} DM of diets). Diets were formulated to be isonitrogenous, including 100 g kg^{-1} forage (chopped fresh sugarcane) (Table 1). The crude glycerin had 1.27 g/cm^3 of density, 899.8 g kg^{-1} DM, 11.9 g ether extract (EE) kg^{-1} DM, 78.6 g mineral matter kg^{-1} DM, 803.5 g glycerol kg^{-1} DM, 74.7 g sodium chloride kg^{-1} DM, and < 0.1 g methanol kg^{-1} DM.

Prior to the feedlot period, the animals were dewormed and supplied with ADE vitamins. The experiment lasted 104 days, including the initial 14 days for adaptation of animals to facilities and diets, and the remaining 90 days for data collection. Animals were weighed at the beginning and end of the experimental period after total fasting for 14-16 h. Average daily gain was calculated as:

$$\text{ADG (kg day}^{-1}) = [(\text{final body weight; FBW}) - \text{initial body weight}]/90 \text{ days}$$

Animals were fed at 10:00 h *ad libitum*, allowing leftover of 100 g kg^{-1} in relation to the feed offered. Intake was monitored daily through the weighing of feed and leftovers from the previous day. Feed efficiency (FE) was calculated as:

$$\text{ADG/DM intake (DMI; kg day}^{-1})$$

Apparent digestibility was evaluated at the end of the experimental period. The fecal grab samples from each animal were collected for three consecutive days (at 09.00 h on the first day, at 13.00 h on the second day, and at 17.00 h on the third day). Fecal samples were pre-dried in a forced-air oven at 55 °C for 72 h and ground in a mill with 2-mm screen sieves. Fecal output was estimated using indigestible neutral detergent fiber (iNDF) as an internal marker (Cochran et al., 1986). To determine iNDF, samples of feces, feeds, and orts were incubated in the rumen of a fistulated cow for a period of 240 h according to technique described by Casali et al. (2008). The fecal output was calculated as:

$$\text{Fecal output (g DM day}^{-1}) = \text{iNDF intake (kg day}^{-1})/\text{iNDF (g g DM}^{-1}) \text{ in faeces}$$

Apparent digestibility (AD) of nutrients was calculated as:

$$\text{AD (kg kg}^{-1}) = [(\text{nutrient intake (kg)} - \text{nutrients excreted (kg)})/(\text{nutrient intake (kg)})]\times 100$$

Samples of ingredients of diets and orts from each animal were collected weekly, and from each two sample, a composite sample was made and stored. All samples were pre-dried in a forced-air oven at 55 °C for 72 h and ground through a mill with 1-mm screen sieves. Standard procedures of AOAC (2000) were adopted when calculating the following components from feed, leftovers, and fecal samples: DM (Reference 930.15), mineral matter (MM; Reference 942.05), crude protein (CP) (Reference 984.13), and EE (Reference 920.39). Neutral detergent fiber and acid detergent fiber (ADF) were measured according to Van Soest et al. (1991). Total carbohydrates (TC) and non-fibrous carbohydrates (NFC) were estimated as described by Sniffen et al. (1992), wherein TC = 100 − (CP + EE + MM) and NFC = 100 −(TC + NDF). The apparent digestible energy intake (DEI) was calculated using the caloric coefficients of nutrients (17.6 MJ kg^{-1} for TC, 24 MJ kg^{-1} for protein, and 39 MJ kg^{-1} for fat), as follows (CSIRO, 2007):

$$\text{DEI (MJ day}^{-1}) = (\text{digestible CP intake} \times 24) + (\text{digestible EE intake} \times 39) + (\text{digestible NDF intake} \times 17.6) + (\text{digestible NFC intake} \times 17.6)$$

The concentration of digestible energy of the DM intake (DE, MJ kg^{-1} DM) was determined by dividing DEI by DM intake (DMI).

Blood samples were collected during the last weighing of the experimental period by venous puncture of the jugular vein using vacutainer tubes (Labtest Diagnóstica SA, Brazil). To determine the glucose concentrations, blood was collected in tubes containing sodium fluoride. For the other analyses, the blood was collected in tubes with potassium EDTA as an anticoagulant. Blood samples were cooled and

Table 1 - Ingredients and chemical composition of diets (dry matter basis)

Item		Glycerin level in the diets (g kg^{-1})			
		0	80	160	240
Ground corn		743	652	552	451
Soybean meal		113	130	149	169
Sugarcane		95.9	95.9	96.1	96.1
Crude glycerin		-	80	160	241
Limestone		22	19	18	18
Mineral mixture[1]		12	13	14	14
Sodium chloride[2]		3.0	-	-	-
Urea		10	10	10	10
Ammonia sulphate		1.0	1.0	1.0	1.0
Rumensin™		0.2	0.2	0.2	0.2
			Nutrient (g kg^{-1} DM)		
	Sugarcane				
Dry matter	268	802	782	763	754
Ash	20.2	20.7	26.6	32.6	38.70
Crude protein	22.7	151	151	154	156
Ether extract	10.3	19.2	22.7	23.6	24.1
Neutral detergent fiber	493	169	162	151	147
Acid detergent fiber	283	69	65	61	60
Total carbohydrates	947	932	932	932	932
Non-fibrous carbohydrates	460	682	681	681	679

[1] Mineral mixture composition: Ca (max), 269 g kg^{-1}; Ca (min), 220 g kg^{-1}; P (min), 160 g kg^{-1}; Mg, 10 g kg^{-1}; S, 15 g kg^{-1}; Zn, 5472 mg kg^{-1}; Fe, 2610 mg kg^{-1}; Cu, 2100 g kg^{-1}; Mn, 992 mg kg^{-1}; Co, 200 mg kg^{-1}; I, 124 mg kg^{-1}; Se, 45 mg kg^{-1}; F (max), 1476 mg; soluble phosphorus in 2% citric acid (max), 900 g kg^{-1}; Rumensin™, 10 g 100 g^{-1} of monensin.
[2] Sodium chloride (40 g kg^{-1} of sodium).

centrifuged at 2,000 × *g* for 20 min at 37 °C. Next, the serum was separated by vacuum suction, divided into aliquots, and placed in labeled lidded plastic Eppendorf® tubes that were frozen (−20 °C) for later biochemical analyses. Serum biochemical analyses of triglycerides, total cholesterol (TCH), high-density lipoprotein (HDL), total protein (TP), urea, albumin, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glucose were performed at 37 °C using a commercial kit (Labtest Diagnóstica SA, Brazil). Readings were performed by using a spectrophotometer (Bioplus® model Bio-2000 IL-A).

At six months of age, the animals were slaughtered in a commercial slaughterhouse. The carcasses were weighed followed by washing and cooling in a cold room at 2 °C for 24 h. Afterwards, they were weighed again. Chilling loss (CL) was calculated as:

$$CL\ [g\ kg^{-1}\ of\ hot\ carcass\ weight\ (HCW)] = [HCW - cold\ carcass\ weight\ (CCW)/HCW]$$

Subcutaneous fat thickness and *longissimus dorsi* area (LDA) were determined between the 12th and 13th ribs with the aid of a caliper and ImageJ™ software, respectively. The weight of the internal organs (reticulorumen, omasum, abomasum, small intestine, large intestine, heart, kidneys, liver, and lungs) without their contents was obtained, and then expressed as g kg^{-1} of empty body weight (EBW). The EBW was determined by the sum of the HCW, internal organs weights, and weights of others non-carcass components (spleen, internal fats, blood, head, feet, leather, and tail switch). Hot carcass yield (HCY) was calculated as: HCY (kg kg^{-1} EBW) = HCW/EBW. Cold carcass yield (CCY) was calculated as: CCY (kg kg^{-1} EBW) = CCW/EBW. The physical composition of the carcass was estimated according to Hankins and Howe (1946) and expressed as kg kg^{-1} of CCW.

Samples of the cranial portion of the ventral coronary pillar in the ventral sac of the rumen were removed with a scalpel and preserved in a 10% (v/v) formaldehyde solution. The samples were later evaluated for height, width, and area of the papilla through image analysis digitalized with ImageJ™ software. Rumen wall thickness (lamina propria mucosae, muscularis, and serosa) was determined using a stereoscopic microscope and millimeter ruler.

Data were subjected to analysis of variance and contrast by mixed model methodology (Littell et al., 2006). The mathematical model is represented by:

$$Y_{ijk} = \mu + T_i + M_j + e_{ijk},$$

in which μ = overall mean, T_i = effect of the diets, M_j = effect of the covariate (initial body weight), and e_{ijk} = residual random error. For probability of type I error, $\alpha = 0.05$. The statistical analysis was conducted with the aid of SAS software (Statistical Analysis System, version 9.1).

3. Results

The intake of DM, CP, NDF, NFC, and DE decreased linearly ($P<0.05$) with increasing levels of crude glycerin in the diets (Table 2). The apparent digestibility coefficients of DM and other nutrients were similar ($P>0.05$) among diets (Table 2). Similarly, ADG, FE, and FBW did not change ($P>0.05$) with diets.

The levels of crude glycerin in diets did not affect ($P>0.05$) serum glucose, triglycerides, cholesterol, high-density lipoprotein (HDL), TP, albumin, urea, ALT, and AST concentrations (Table 3).

The EBW, HCW, CCW, HCY, CCY, CL, subcutaneous fat thickness (SFT), LDA, and carcass tissue composition were similar ($P>0.05$) among diets (Table 4).

Diets did not influence ($P>0.05$) weights (g kg^{-1} EBW) of lungs, heart kidneys, liver, omasum, abomasum, and large intestine (Table 5). Reticulorumen weight (g kg^{-1} EBW) increased ($P<0.05$) and the small intestine weight (g kg^{-1} EBW) decreased with increasing levels of crude glycerin. The area, height, and width of rumen papillae were not changed ($P>0.05$), but the thickness of rumen wall increased ($P<0.05$) with increasing levels of crude glycerin.

Table 2 - Nutrient intake and apparent digestibility of diets and performance of dairy calves fed crude glycerin levels

Variable	Glycerin level (g kg^{-1})				SE	P-value	
	0	80	160	240		L	Q
			Intake				
DM[1] (kg day^{-1})	4.14	4.11	3.80	3.49	0.36	0.002	0.952
DM[2] (g kg^{-1} BW)	29.6	28.4	25.5	24.3	2.20	<0.001	0.837
NDF[3] (g kg^{-1} BW)	5.1	4.7	4.0	4.1	0.50	0.001	0.286
NFC[4] (g kg^{-1} BW)	19.7	19.0	16.7	15.7	1.40	<0.001	0.985
CP (g kg^{-1} BW)	4.4	4.3	4.0	3.9	0.33	0.010	0.8953
DEI (MJ kg^{-1} BW)	0.43	0.41	0.37	0.35	0.03	<0.001	0.458
DE (MJ kg^{-1} DM)	14.4	14.5	14.5	14.6	0.16	0.341	0.899
			Apparent digestibility				
DM	0.73	0.72	0.73	0.74	2.53	0.202	0.372
CP	0.78	0.77	0.78	0.79	8.18	0.669	0.737
NDF	0.38	0.37	0.38	0.39	3.26	0.563	0.656
NFC	0.85	0.84	0.85	0.86	2.22	0.313	0.235
			Animal performance				
IBW (kg)	91.0	94.2	98.6	98.1	11.8	-	-
FBW (kg)	195	195	199	194	16.1	0.808	0.952
ADG (kg day^{-1})	1.12	1.09	1.11	1.03	0.16	0.592	0.940
FE (kg kg^{-1})	0.28	0.27	0.30	0.30	0.04	0.166	0.948

DM - dry matter intake ([1]$\hat{Y}$ = 4.1988 – 0.0299x, R^2 = 0.39; [2]$\hat{Y}$ = 29.739 – 0.239x, R^2 = 0.52); NDF - neutral detergent fiber ([3]$\hat{Y}$ = 5.037 – 0.048x, R^2 = 0.42); NFC - non-fibrous carbohydrates ([4]$\hat{Y}$ = 19.859 – 0.181x, R^2 = 0.60); CP - crude protein intake ($\hat{Y}$ = 4.385 – 0.022x; R^2 = 0.29); DEI - apparent digestible energy intake ($\hat{Y}$ = 0.428 – 0.003x, R^2 = 0.96); DE - concentration of digestible energy of the DM intake; IBW - initial body weight; FBW - final body weight; ADG - average daily gain; FE - feed efficiency; SE - standard error; L - linear effect; Q - quadratic effect.

Table 3 - Blood parameters of dairy calves fed crude glycerin levels

Variable	Glycerin level (g kg^{-1})				SE	P-value	
	0	80	160	240		L	Q
Glucose (mg dL^{-1})	105.11	101.04	107.34	103.61	15.07	0.952	0.982
Triglycerides (mg dL^{-1})	122.23	127.25	127.81	122.41	45.66	0.721	0.067
Cholesterol (mg mL^{-1})	103.95	114.68	113.48	103.60	25.85	0.968	0.159
HDL (mg mL^{-1})	81.10	80.41	80.59	80.43	15.47	0.865	0.923
Total protein (g dL^{-1})	4.83	4.78	5.20	4.86	1.02	0.633	0.489
Albumin (g dL^{-1})	3.54	3.51	3.51	3.52	0.27	0.910	0.868
Urea (mg dL^{-1})	28.46	28.98	28.40	27.89	3.98	0.587	0.598
ALT (U L^{-1})	13.72	16.13	16.31	15.74	12.65	0.512	0.515
AST (U L^{-1})	17.86	17.75	15.44	15.11	11.16	0.140	0.388

HDL - high-density lipoprotein; ALT - alanine aminotransferase; AST - aspartate aminotransferase; SE - standard error; L - linear effect; Q - quadratic effect.

4. Discussion

We hypothesized that partial replacement of corn grain with crude glycerin would not compromise DMI and apparent digestibility. However, compared with corn grain, crude glycerin decreased intakes of DM, CP, NDF, and NFC in diets of dairy calves. Nevertheless, the concentration of DE of the DMI was similar among diets (Table 2). This was due to higher energy levels for glycerin compared with corn grain, in which glycerin contributes with more energy per unit of DM than corn grain (Monnerat et al., 2013; Benedeti et al., 2016).

The reduction in DMI has been observed in cattle fed crude glycerin, and the literature indicates possible reasons for this observation ranging from effects on ruminal metabolism up to effects on

intermediary metabolism. It is well known that crude glycerin has the capacity of increasing propionate concentration in the rumen (Wang et al., 2009; Lee et al., 2011; Vongsamphanh et al., 2017). The reduction in DMI might be due to hepatic oxidation stimulated by propionate. Propionate is likely a primary satiety signal, because its flux to the liver increases greatly during meals and because of its high hepatic extraction in the portal vein (Allen, 2000).

Other studies, however, have reported that DMI was not affected by inclusion of crude glycerin. Mach et al. (2009) observed no effect on DMI in Holstein bulls fed four levels of crude glycerin (0, 40, 80, and 120 g kg^{-1} DM of concentrate). Likewise, Barros et al. (2018) did not observe a reduction on DMI when crude glycerin was added up to 240 g kg^{-1} in finisher diets for Holstein-Zebu crossbred bulls. Maciel et al. (2016a), on the other hand, verified a linear increase in the DMI of dairy crossbred veal calves fed starter concentrate containing crude glycerin at 0, 80, 160, and 240 g kg^{-1} DM , which was attributed to the fact that crude glycerin is viscous, hygroscopic, and has a relatively sweet flavor, characteristics that, according these authors, increase the palatability of diets.

Table 4 - Carcass characteristics of dairy calves fed crude glycerin levels

Variable	Glycerin level (g kg^{-1})				SE	P-value	
	0	80	160	240		L	Q
Empty body weight (kg)	167.40	171.30	178.60	172.60	16.40	0.451	0.707
Hot carcass weight (kg)	97.70	97.80	99.10	96.90	9.40	0.944	0.355
Cold carcass weight (kg)	95.00	94.60	96.90	94.40	9.40	0.976	0.707
Hot carcass yield (kg kg^{-1} EBW)	0.58	0.57	0.56	0.56	1.70	0.770	0.421
Cold carcass yield (kg kg^{-1} EBW)	0.57	0.55	0.54	0.54	1.70	0.158	0.356
Chilling loss (g kg^{-1} HCW)	27.63	32.72	22.20	25.80	1.10	0.437	0.991
Subcutaneous fat thickness (mm)	1.00	0.80	0.87	0.78	0.38	0.573	0.900
Longissiums dorsi area (cm^2)	44.82	44.81	47.11	44.21	3.87	0.861	0.952
Muscle (kg kg^{-1} CCW)	66.8	65.1	66.8	65.2	2.13	0.566	0.987
Bone (kg kg^{-1} CCW)	16.8	18.1	16.7	18.6	1.87	0.221	0.538
Fat (kg kg^{-1} CCW)	16.4	16.3	16.6	15.6	1.97	0.173	0.376

EBW - empty body weight; HCW - hot carcass weight; CCW - cold carcass weight; SE - standard error; L - linear effect; Q - quadratic effect.

Table 5 - Internal organ characteristics of dairy calves fed crude glycerin levels

Variable	Glycerin level (g kg^{-1})				SE	P-value	
	0	80	160	240		L	Q
Lung (g kg^{-1} EBW)	12.30	13.90	10.70	12.20	1.80	0.262	0.893
Heart (g kg^{-1} EBW)	5.50	5.60	5.60	5.30	0.50	0.156	0.348
Kidneys (g kg^{-1} EBW)	3.90	4.00	3.40	4.00	0.60	0.655	0.215
Liver (g kg^{-1} EBW)	20.10	20.80	19.90	20.20	2.30	0.869	0.409
Reticulorumen (g kg^{-1} EBW)[1]	23.80	25.10	25.60	26.00	1.00	0.001	0.707
Omasum (g kg^{-1} EBW)	6.00	5.90	6.80	6.30	1.60	0.791	0.649
Abomasum (g kg^{-1} EBW)	5.20	5.10	5.80	5.90	0.80	0.218	0.770
Large intestine (g kg^{-1} EBW)	11.80	13.50	11.80	11.10	2.50	0.300	0.995
Small intestine (g kg^{-1} EBW)[2]	19.30	18.90	17.80	16.60	1.40	0.002	0.707
Papillae area (mm^2)	20.00	24.80	21.00	25.00	4.26	0.488	0.986
Papillae length (mm)	5.60	6.50	6.10	6.50	1.45	0.374	0.697
Papillae width (mm)	2.40	2.50	2.30	2.60	0.50	0.488	0.614
Rumen wall thickness (μm)[3]	1230	1292	1642	1685	168	0.001	0.910

EBW - empty body weight; SE - standard error; L - linear effect; Q - quadratic effect.
[1] Reticulorumen ($\hat{Y}$ = 22.45 + 0.0011x; R^2 = 0.41)
[2] Small intestine ($\hat{Y}$ = 19.5 – 0.011x, R^2 = 0.36).
[3] Rumen wall thickness ($\hat{Y}$ = 1204.58 + 2.14x, R^2 = 0.60).

The reduction in CP intake by inclusion of crude glycerin was possibly associated to the reduction of DMI, since the diets presented similar CP content (Table 1). On the other hand, diets with greater levels of crude glycerin had lower levels of NDF and NFC, which associated with the decrease of DMI, explain the reduction on intake of NDF and NFC with inclusion of crude glycerin. The results of this study were consistent with the literature, since the reduction of nutrients of the diets and DMI decrease determined lower intake of nutrients by inclusion of crude glycerin in diets (Hales et al., 2013; Benedeti et al., 2016).

The similar apparent digestibility of diets containing crude glycerin is well characterized in the literature (Avila-Stagno et al., 2013; Benedeti et al., 2016; Maciel et al., 2016a). However, Barros et al. (2018) and Saleem and Singer (2018) reported a negative influence of glycerol on NDF digestion, which has been attributed to the decrease in the populations of fibrolytic bacteria. In this study, it is possible that grain-based diets suppressed the activity of cellulolytic bacteria, which may have minimized the effect of crude glycerin on NDF apparent digestibility. In addition, the NDF content in the grain-based diets is naturally low, which possibly also contributed to the NDF digestibility results.

The similar animal performance (ADG and FBW) among diets, considering the DMI reduction, could be associated with the similar concentration of DE intake among diets. On the other hand, the similar FE was possibly associated with the similar ADG among diets (Table 2). Glycerin-containing diets improve the energy use efficiency of the animal due to the lower loss of energy as methane, and the energy retained may be directed to tissue gain (Lee et al., 2011). Glycerin promotes increase in the uptake of gluconeogenic substances (especially propionate) and enhances the energy efficiency gain (DeFrain et al., 2004). The intermediate metabolism of glycerol is also more efficient because, after being absorbed through the intestine and/or through the rumen wall (Donkin et al., 2009), it is converted to glycerol-3-phosphate and ADP in the liver (DeFrain et al., 2004). This is an intermediary stage of glycolysis, in which glycerol can be directed both to glycolysis or gluconeogenesis. This process of glycerol metabolism has the advantage of not having its regulation limited by the pyruvate carboxylase and phosphoenolpyruvate carboxylase enzymes, because it enters the gluconeogenic pathway at the triose phosphate level, which is metabolically closer to glucose (DeFrain et al., 2004).

The presented results were similar to those obtained by Benedeti et al. (2016), who evaluated levels of crude glycerin (0, 50, 100, and 150 g kg^{-1} DM basis) and found no difference in ADG, FBW, and feed conversion (FC) of Nellore bulls. Leão et al. (2012) also did not find alterations of ADG of beef cattle with levels of crude glycerin increased to up to 240 g kg^{-1} DM basis. Similarly, Van Cleef et al. (2014) did not find alterations in the ADG and FC of beef cattle with increasing levels of crude glycerin up to 300 g kg^{-1} DM. On the other hand, Maciel et al. (2016a) verified elevation of ADG and FBW and decrease of FC of lactating dairy calves with increasing level of crude glycerin, which was attributed to the increase in DMI. Similarly, Moreira et al. (2016) and Barros et al. (2018) verified elevation of ADG and decrease of FC of Nellore bulls and crossbreed dairy bulls (respectively) fed levels of crude glycerin (0, 60, 120, and 240 g kg^{-1} DM), which was attributed to the increase in FE. Lage et al. (2010), on the other hand, found a decrease in ADG of Santa Inês lambs with inclusion of crude glycerin (0, 30, 60, 90, and 120 g kg^{-1} DM) in the diets, which was associated with reduced DMI due to the high content of fatty acids in crude glycerin. According to Moreira et al. (2016), this variability of animal responses for the inclusion of crude glycerin in ruminant diets may be associated with species and/or animal categories, level of concentrate, levels of crude glycerin, type of feedstuff replaced by crude glycerin, and composition and nutritional value of the crude glycerin utilized in the experimental diets.

The increase in energy efficiency with increased levels of crude glycerin may have been determinants for similar glucose content among diets (Table 3). The glucose values obtained, on the other hand, were higher than those considered normal (45-75 mg dL^{-1}) for cattle (González and Silva, 2006), which may be associated with the animal category utilized in this experiment. Animal age is an important consideration when interpreting blood glucose concentration, because younger animals have higher values than adult animals (Mohri et al., 2007). The higher blood glucose values in young animals are related to high activity of liver enzymes responsible for glucose release and greater plasma concentrations of growth hormone to support rapid growth (Mondal and Prakash, 2004).

The high glucose concentration might have also contributed to the high levels of total cholesterol, considering that 46.3 to 79.7 mg dL^{-1} is recommended for calves weaned between 3 and 12 months of age (Pogliani and Birgel Junior, 2007). These higher values are likely linked to increases in lipogenesis and reduction of lipolysis stimulated by insulin in the adipose tissue (French and Kennelly, 1990; Thrall et al., 2006). The HDL showed an age effect similar to that of total cholesterol, which is explained by HDL being the main form of transference of cholesterol from the liver and small intestine to the peripheral tissues. The HDL found in calves agreed with the 81.22±30.06 mg L^{-1} reported by Osorio et al. (2012) for younger males.

The measurement of TP, albumin, and globulin are important for the diagnosis of diseases and disorders in the liver. Despite being below the recommended reference interval of 5-7 g dL^{-1}, the plasma concentration of TP and albumin could be considered normal for calves at 3-5 months of age (Lohakare et al., 2006). Young animals typically display lower TP and albumin values than adult animals (Doornenbal et al., 1988). In addition, transaminase activity occurred within the range considered normal for the species (Meyer and Marvey, 1998), demonstrating that there were no lesions or damage to the liver. Likewise, blood urea levels remained within the interval of 20 to 30 mg dL^{-1} (Kaneko et al., 1977), suggesting normal renal function.

The similar carcass characteristics among levels of crude glycerin can be explained by the animal performance, which indicates similar body development. The results of the present study were consistent with those verified by Benedeti et al. (2016), who did not find changes in HCW, HCY, carcass average daily gain, LDA, and SFT in Nellore bulls fed levels of crude glycerin, which was attributed to the similar energy intake, CP intake, and ADG among diets. Similarly to our results, no effects on carcass characteristics were reported when crude glycerin was included at up to 100 g kg^{-1} DM in diets of finishing bulls (Bartoň et al., 2013; Lage et al., 2014). Maciel et al. (2016b) also observed a lack of effects in HCW, CCW, HCY, CCY, SFT, LDA, and carcass physical composition when crude glycerin was included at up to 240 g kg^{-1} DM basis in diets of dairy steers finished in feedlot. Moreover, improved HCW and LDA were observed when bulls (Françozo et al., 2013) and beef calves (Gunn et al., 2011) fed diets containing 120 and 150 g kg^{-1} DM crude glycerin, respectively, suggesting that the inclusion of this ingredient can increase muscle growth in feedlot finishing cattle by the increase in energy efficiency.

The weight of internal organs, according to Dias et al. (2016), follows body weight in dairy calves, which may explain much of the results for internal organ characteristics. In addition, according Perón et al. (1993), heart and lungs maintain their integrity because they have a priority in nutrient use, regardless of the intake level. However, some change in liver weight was expected with increasing levels of crude glycerin because this is the main organ responsible for metabolizing absorbed glycerol (Donkin, 2008). However, as verified from the blood parameters, there was apparently no effect of the diets on the metabolic activity of these organs. It is possible that grain-based diets led to high metabolic activity in these organs, making it difficult to verify the effect of the inclusion of crude glycerin on the weight of this organ.

An increase in the weight of the reticulorumen may occur because of increased rumen mass and papillae growth (Khan et al., 2007). No significant differences were found with respect to the area, height, and width of the rumen papillae with the inclusion of crude glycerin in the diets. Thus, the increased reticulorumen weight is associated with increased rumen wall thickness, which, according to Daniel and Resende Júnior (2012), is associated with the metabolism of fatty acids (especially butyrate) in this organ. The inclusion of crude glycerin in diet of ruminants, in this context, has been responsible for increasing the proportion of propionate and ruminal butyrate (Mondal and Prakash, 2004; Vongsamphanh et al., 2017).

The decrease in the weight of the small intestine, however, can be explained by DMI reduction, which could have reduced metabolic activity in this organ, altering its size (Missio et al., 2009b). In general, it has been verified that the weight of the large intestine, on the other hand, is not altered by the supply of different diets and/or feeding systems (Dias et al., 2016), which possibly is associated with less metabolic activity in relation to small intestine, which has the absorption of water as one of the main functions (Gasaway et al., 1976).

5. Conclusions

The inclusion up to 240 g kg^{-1} DM of crude glycerin in corn grain-based diets for the production of dairy calves does not alter animal performance, carcass characteristics, and weights of internal organs.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: R.P. Maciel and J.N.M. Neiva. Data curation: R.P. Maciel. Formal analysis: R.P. Maciel and L.F. Sousa. Funding acquisition: J.N.M. Neiva. Investigation: R.P. Maciel, J. Restle, R.L. Missio, U.O. Bilego, M.S. Cunha, V.L. Araújo and J.N.M. Neiva. Methodology: R.P. Maciel, J. Restle, V.L. Araújo and J.N.M. Neiva. Supervision: J. Restle and J.N.M. Neiva. Writing-original draft: R.P. Maciel, J. Restle and J.N.M. Neiva. Writing-review & editing: R.P. Maciel and R.L. Missio.

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