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. 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

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/cm³ 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:

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

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:

\[
\frac{\text{ADG}}{\text{DM intake (DMI; kg day}^{-1}\text{)}}
\]

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

Apparent digestibility (AD) of nutrients was calculated as:

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

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):

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

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 Diagnostica 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\(^\text{™}\) |                                           | 0.2| 0.2| 0.2 | 0.2 |

| Nutrient (g kg\(^{-1}\) DM) |
|-----------------------------|
| Sugarcane                   |
| Dry matter                  | 268                           |
| Ash                         | 20.2                          |
| Crude protein               | 22.7                          |
| Ether extract               | 10.3                          |
| Neutral detergent fiber     | 493                           |
| Acid detergent fiber        | 283                           |
| Total carbohydrates         | 947                           |
| Non-fibrous carbohydrates   | 460                           |

\(^1\) 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 mg 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\(^\text{™}\), 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:

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

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:

\[
Y_{ij} = \mu + T_i + M_j + e_{ij}
\]

in which \(\mu\) = overall mean, \(T_i\) = effect of the diets, \(M_j\) = effect of the covariate (initial body weight), and \(e_{ij}\) = 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.
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

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 |
|------------------------|-------------------------|----|---------|
| Intake                 |                         |    |         |
| DM (kg day⁻¹)          | 4.14                    | 4.11| 3.80    | 3.49| 0.36| 0.002| 0.952|
| DM (g kg⁻¹ BW)         | 29.6                    | 28.4| 25.5    | 24.3| 4.0| 4.1| 0.50| <0.001| 0.837|
| NDF (g kg⁻¹ BW)        | 5.1                     | 4.7 | 4.0     | 4.1 | 0.43| 0.43| 0.37| 0.35| 0.03| <0.001| 0.458|
| NFC (g kg⁻¹ BW)        | 19.7                    | 19.0| 16.7    | 15.7| 4.4| 4.3| 4.0| 3.9| 0.33| 0.010| 0.8953|
| CP (g kg⁻¹ BW)         | 4.4                     | 4.3 | 4.0     | 3.9 | 4.4| 4.3| 4.0| 3.9| 0.33| 0.010| 0.8953|
| DEI (MJ kg⁻¹ DM)       | 0.43                    | 0.41| 0.37    | 0.35| 0.43| 0.41| 0.37| 0.35| 0.03| <0.001| 0.458|
| DEI (MJ kg⁻¹ DM)       | 14.4                    | 14.5| 14.5    | 14.6| 14.4| 14.5| 14.5| 14.6| 0.16| 0.341| 0.899|

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

| Variable               | Glycerin level (g kg⁻¹) | SE | P-value |
|------------------------|-------------------------|----|---------|
| 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| 127.81| 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| 0.686|
| 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|

Table 4 - Summary of the data and performance of dairy calves fed crude glycerin levels

| Variable               | Glycerin level (g kg⁻¹) | SE | P-value |
|------------------------|-------------------------|----|---------|
| Intake                 |                         |    |         |
| DM (kg day⁻¹)          | 4.14                    | 4.11| 3.80    | 3.49| 0.36| 0.002| 0.952|
| DM (g kg⁻¹ BW)         | 29.6                    | 28.4| 25.5    | 24.3| 4.0| 4.1| 0.50| <0.001| 0.837|
| NDF (g kg⁻¹ BW)        | 5.1                     | 4.7 | 4.0     | 4.1 | 0.43| 0.43| 0.37| 0.35| 0.03| <0.001| 0.458|
| NFC (g kg⁻¹ BW)        | 19.7                    | 19.0| 16.7    | 15.7| 4.4| 4.3| 4.0| 3.9| 0.33| 0.010| 0.8953|
| CP (g kg⁻¹ BW)         | 4.4                     | 4.3 | 4.0     | 3.9 | 4.4| 4.3| 4.0| 3.9| 0.33| 0.010| 0.8953|
| DEI (MJ kg⁻¹ DM)       | 0.43                    | 0.41| 0.37    | 0.35| 0.43| 0.41| 0.37| 0.35| 0.03| <0.001| 0.458|
| DEI (MJ kg⁻¹ DM)       | 14.4                    | 14.5| 14.5    | 14.6| 14.4| 14.5| 14.5| 14.6| 0.16| 0.341| 0.899|

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 | 0.494 | 0.555 |
| 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 |

EBW - empty body weight; HCW - hot 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) | 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) | 19.30      | 18.90 | 17.80 | 16.60 | 1.40 | 0.002 | 0.707 |
| Papillae area (mm²)       | 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) | 12.30      | 12.92 | 16.42 | 16.85 | 1.68 | 0.001 | 0.910 |

EBW - empty body weight; SE - standard error; L - linear effect; Q - quadratic effect.
1 Reticulorumen (Ŷ = 22.45 + 0.0011x; R² = 0.41)
2 Small intestine (Ŷ = 19.5 - 0.011x, R² = 0.36).
3 Rumen wall thickness (Ŷ = 1204.58 + 2.14x, R² = 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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References

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83:1598-1624. https://doi.org/10.3168/jds.S0022-0302(00)75030-2

Abu Bakar, C.; Moh Salleh, R. and Mohamad Shafit, H. 2001. Effect of milk diets on growth performance and carcass composition as veal from Sahiwal-Friesian calves. Journal of Tropical Agriculture and Food Science 29:247-251.

AOAC - Association of Official Analytical Chemists. 2000. Official methods of analysis. 17th ed. AOAC International, Arlington, VA.

Avila-Stagno, J.; Chaves, A. V.; He, M. L.; Harstad, O. M.; Beauchemin, K. A.; McGinn, S. M. and McAllister, T. A. 2013. Effects of increasing concentrations of glycerol in concentrate diets on nutrient digestibility, methane emissions, growth, fatty acid profiles, and carcass traits of lambs. Journal of Animal Science 91:829-837. https://doi.org/10.2527/jas.2012-5215

Barros, A. C. B.; Neiva, J. N. M.; Restle, J.; Missio, R. L.; Miotto, F. R. C.; Elejalde, D. A. G. and Maciel, R. P. 2018. Production responses in young bulls fed glycerin as a replacement for concentrates in feedlot diets. Animal Production Science 58:856-861. https://doi.org/10.1071/AN16288

Bartoň, L.; Bureš, D.; Homolka, P.; Jančík, F.; Marounek, M. and Řehák, D. 2013. Effects of long-term feeding of crude glycerine on performance, carcass traits, meat quality, and blood and rumen metabolites of finishing bulls. Livestock Science 155:53-59. https://doi.org/10.1016/j.livsci.2013.04.010

Benedeti, P. D. B.; Paulino, P. V. R.; Marcondes, M. I.; Maciel, I. F. S.; Silva, M. C. and Faciola, A. P. 2016. Partial replacement of ground corn with glycerol in beef cattle diets: intake, digestibility, performance, and carcass characteristics. Plos One 11:e0148224. https://doi.org/10.1371/journal.pone.0148224

Casali, A. O.; Detmann, E.; Valadares Filho, S. C.; Pereira, J. C.; Henriques, L. T.; Freitas, S. G. and Paulino, M. F. 2008. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos in situ. Revista Brasileira de Zootecnia 37:335-342. https://doi.org/10.1590/S1516-35982008000200021

Cochran, R. C.; Adams, D. C.; Wallace, J. D. and Galvean, M. L. 1986. Predicting digestibility of different diets with internal markers: evaluation of four potential markers. Journal of Animal Science 63:1476-1483. https://doi.org/10.2527/jas1986.631476x

Daniel, J. L. P. and Resende Júnior, J. C. 2012. Absorption and metabolism of volatile fatty acids by rumen and omasum. Ciência e Agrotecnologia 36:93-99. https://doi.org/10.1590/S1413-70542012000100012

DeFrain, J. M.; Huppen, A. R.; Kalscheur, K. F. and Jardon, P. W. 2004. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. Journal of Dairy Science 87:4195-4206. https://doi.org/10.3168/jds.S0022-0302(04)73564-X
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Maciel et al.

Dias, A. M. O.; Menezes, L. F. G.; Silveira, M. F.; Paris, W.; Santos, P. V. and Lazzarotto, E. F. C. O. 2016. Organs and carcass non-integrant components of Holstein calves, slaughtered at different weights, held in different finishing systems. Semina: Ciências Agrárias 37:1045-1056.

Donkin, S. S.; Koser, S. L.; White, H. M.; Doane, P. H. and Cecava, M. J. 2009. Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows. Journal of Dairy Science 92:5111-5119. https://doi.org/10.3168/jds.2009-2201

Donkin, S. S. 2008. Glycerol from biodiesel production: the new corn for dairy cattle. Revista Brasileira de Zootecnia 37:280-286. https://doi.org/10.1590/S1516-35982008001300032

Doornenhal, H.; Tong, A. K. W. and Murray, N. L. 1988. Reference values of blood parameters in beef cattle of different ages and stages of lactation. Canadian Journal of Veterinary Research 52:99-105.

Françozo, M. C.; Prado, I. N.; Cecato, U.; Valero, M. V.; Zawadzki, F.; Ribeiro, O. L.; Prado, R. M. and Visentainer, J. V. 2013. Growth performance, carcass characteristics and meat quality of finishing bulls fed crude glycerin-supplemented diets. Brazilian Archives of Biology and Technology 56:327-336. https://doi.org/10.1590/S1516-89132013000200019

French, N. and Kenemly, J. J. 1990. Effects of feeding frequency on ruminal parameters, plasma insulin, milk yield, and milk composition in Holstein cows. Journal of Dairy Science 73:1857-1863. https://doi.org/10.3168/jds.S0022-0302(90)78866-2

Gasaway, W. C.; White, R. G. and Holleman, D. F. 1976. Digestion of dry matter and absorption of water in the intestine and caecum of rock partridge. Condor 78:77-84. https://doi.org/10.2307/1366918

González, F. H. D. and Silva, S. C. 2006. Introdução à bioquímica clínica veterinária. 2.ed. Universidade Federal do Rio Grande do Sul, Porto Alegre. 364p.

CSIRO - Commonwealth Scientific and Industrial Research Organization. 2007. Nutrient requirements of domesticated ruminants. CSIRO Publishing, Melbourne.

Gunn, P. J.; Lemenager, R. P.; Buckmaster, D. R.; Claeyss, M. C. and Lake, S. L. 2011. Effects of dried distillers grains with solubles and crude glycerin on performance, carcass characteristics, and metabolic parameters of early weaned beef calves. The Professional Animal Scientist 27:283-294. https://doi.org/10.15232/S1080-7446(15)30491-5

Hales, K. E.; Bondurant, R. G.; Luebbe, M. K.; Cole, N. A. and MacDonald, J. C. 2013. Effects of crude glycerin in steam-flaked corn-based diets fed to growing feedlot cattle. Journal of Animal Science 91:3875-3880. https://doi.org/10.2527/jas.2012-5944

Hankins, O. G. and Howe, P. E. 1946. Estimation of composition of beef carcases and cuts. USDA - Technical Bulletin, 926. USDA, Washington, DC.

Kaneko, J. J.; Harvey, J. W. and Bruss, M. L. 1977. Clinical biochemistry of domestic animals. Academic Press, California, USA.

Khan, M. A.; Lee, H. J.; Lee, W. S.; Kim, H. S.; Ki, K. S.; Hur, T. Y.; Suh, G. H.; Kang, S. J. and Choi, Y. J. 2007. Structural growth, rumen development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. Journal of Dairy Science 90:3376-3387. https://doi.org/10.3168/jds.2007-0104

Lage, J. F.; Berchielli, T. T.; San Vito, E.; Silva, R. A.; Ribeiro, A. F.; Reis, R. A.; Dallantonio, E. E.; Simonetti, L. R.; Delevatti, L. M. and Machado, M. 2014. Fatty acid profile, carcass and meat quality traits of young Nellore bulls fed crude glycerin replacing energy sources in the concentrate. Meat Science 96:1158-1164. https://doi.org/10.1016/j.meatsci.2013.10.027

Lage, J. F.; Paulino, P. V. R.; Pereira, L. G. R.; Valadares Filho, S. C.; Oliveira, A. S.; Detmann, E.; Souza, N. K. P. and Lima, J. C. M. 2010. Glicerina bruta na dieta de cordeiros terminados em confinamento. Pesquisa Agropecuária Brasileira 45:1012-1020. https://doi.org/10.1590/S0100-204X2010000900011

Lee, S. Y.; Lee, S. M.; Cho, Y. B.; Kam, D. K.; Lee, S. C.; Kim, C. H. and Seo, S. 2011. Glycerol as a feed supplement for ruminants: in vitro fermentation characteristics and methane production. Animal Feed Science and Technology 166-167:269-274. https://doi.org/10.1016/j.anifeedsci.2011.04.070

Leão, J. P.; Neiva, J. N. M.; Restle, J.; Paulino, P. V. R.; Santana, A. E. M.; Miotto, F. R. C. and Missio, R. L. 2012. Consumo e desempenho de bovinos de aptidão leiteira em confinamento alimentados com glicerol. Ciência Animal Brasileira 13:421-428.

Littell, R. C.; Milliken, G. A.; Stroup, W. W.; Wolfinger, R. D. and Schabenberger, O. 2006. SAS® for Mixed models. 2nd ed. SAS Institute Inc., Cary, USA.

Lolahare, J. D.; Pattanaik, A. K. and Khan, S. A. 2006. Effect of dietary protein levels on the performance, nutrient balances, metabolic profile and thyroid hormones of crossbred calves. Asian-Australasian Journal of Animal Sciences 19:1588-1596. https://doi.org/10.5713/ajas.2006.1588

Mach, N.; Bach, A. and Devant, M. 2009. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. Journal of Animal Science 87:632-638. https://doi.org/10.2527/jas.2008-0987

Maciel, R. P.; Neiva, J. N. M.; Restle, J.; Bilego, U. O.; Miotto, F. R. C.; Fontes, A. J.; Fioravanti, M. C. S. and Oliveira, R. A. 2016a. Performance, rumen development, and carcass traits of male calves fed starter concentrate with crude glycerin. Revista Brasileira de Zootecnia 45:309-318. https://doi.org/10.1590/S1806-92902016000600005
Maciel, R. P.; Neiva, J. N. M.; Restle, J.; Miotto, F. R. C.; Sousa, L. F.; Cunha, O. F. R.; Moron, S. E. and Parente, R. R. P. 2016b. Performance and carcass characteristics of dairy steers fed diets containing crude glycerin. Revista Brasileira de Zootecnia 45:677-685. https://doi.org/10.1590/s1806-92902016001100006

Meyer, D. J. and Marvey, J. W. 1998. Veterinary laboratory medicine: interpretation and diagnosis. 2nd ed. Saunders, Philadelphia, PA.

Missio, R. L.; Brondani, I. L.; Freitas, L. S.; Sachet, R. H.; Silva, J. H. S. and Restle, J. 2009a. Desempenho e avaliação econômica da terminação de tourinhos em confinamento alimentados com diferentes níveis de concentrado na dieta. Revista Brasileira de Zootecnia 38:1309-1316. https://doi.org/10.1590/S1516-35982009000700021

Missio, R. L.; Brondani, I. L.; Restle, J.; Silva, J. H. S.; Silveira, M. F. and Silva, V. S. 2009b. Partes não-integrantes da carcaça de tourinhos alimentados com diferentes níveis de concentrado na dieta. Revista Brasileira de Zootecnia 38:906-915. https://doi.org/10.1590/S1516-35982009000800018

Mohri, M.; Sharifi, K. and Eidi, S. 2007. Hematology and serum biochemistry of Holstein dairy calves: Age related changes and comparison with blood composition in adults. Research in Veterinary Science 83:30-39. https://doi.org/10.1016/j.rvsc.2006.10.017

Mondal, M. and Prakash, B. S. 2004. Changes on plasma non-esterified fatty acids, glucose and α-amino nitrogen and their relationship with body weight and plasma growth hormone in growing buffaloes (Bubalus bubalis). Journal of Animal Physiology and Animal Nutrition 88:223-228. https://doi.org/10.1111/j.1439-0396.2004.00476.x

Monnerat, J. P. I. S.; Oliveira, I. M.; Paulino, P. V. R.; Mezzomo, R.; Silva, L. H. P.; Silva, P. P. and Ferreira, J. P. 2013. Nutritional evaluation and performance of beef cattle fed with crude glycerin diets. p.97-98 In: Energy and protein metabolism and nutrition in sustainable animal production. Oltjen, J.; Kebreab, E.; Lapierre, H., eds. Wageningen Academic Publishers, Wageningen. https://doi.org/10.3920/978-90-8686-781-3-22

Moreira, W. S.; Miotto, F. R. C.; Restle, J.; Missio, R. L.; Neiva, J. N. M. and Moreira, R. V. 2016. Crude glycerin levels in pearl millet-based diets for Nellore young bulls in feedlot. Revista Brasileira de Zootecnia 45:32-38. https://doi.org/10.1590/S1806-92902016000100005

Osorio, J. H.; Vinazco, J. and Pérez, J. E. 2012. Comparación de perfil lipídico por sexo y edad en bovinos. Biosalud 11:25-33.

Pogliani, F. C. and Birgel Junior, E. 2007. Valores de referência do lipidograma de bovinos da raça holandesa, criados no Estado de São Paulo. Brazilian Journal of Veterinary Research and Animal Science 44:373-383. https://doi.org/10.11606/issn.1678-4456.bjvras.2007.26621

Perón, A. J.; Fontes, C. A. A.; Lana, R. P.; Silva, D. J.; Queiroz, A. C. and Paulino, M. F. 1993. Tamanho de órgãos internos e distribuição da gordura corporal, em novilhos de cinco grupos genéticos, submetidos à alimentação restrita e ad libitum. Revista Brasileira de Zootecnia 22:813-819.

Saleem, A. M. and Singer, A. M. 2018. Growth performance and digestion of growing lambs fed diets supplemented with glycerol. Animal 12:959-963. https://doi.org/10.1017/S1751731117001793

Sniffen, C. J.; O’Connor, J. D. O.; Van Soest, P. J.; Fox, D. G. and Russell, J. B. 1992. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. Journal of Animal Science 70:3562-3577. https://doi.org/10.2527/1992.70113562x

Thrall, M. A.; Baker, D. C.; Campbell, T. W; DeNicola, D.; Fettman, M. J.; Lassen, E. D.; Rebar, A. and Weiser, G. 2006. Hematologia e bioquímica clínica veterinária. Roca, São Paulo. 582p.

Vongsamphanh, P.; Preston, T. R. and Leng, R. A. 2017. Glycerol supplementation increased growth rates, decreased the acetate: propionate ratio in rumen VFA, and reduced enteric methane emissions, in cattle fattened on cassava pulp-urea, brewers’ grains and rice straw. Livestock Research for Rural Development 29:Article #036. Available at: <http://www.lrrd.org/lrrd29/2/phan29036.html>. Accessed on: May 28, 2018.

Wang, C.; Liu, Q.; Huo, W. J.; Yang, W. Z.; Dong, K. H.; Huang, Y. X. and Guo, G. 2009. Effects of glycerol on rumen fermentation, urinary excretion of purine derivatives and feed digestibility in steers. Livestock Science 121:15-20. https://doi.org/10.1016/j.livsci.2008.05.010