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# Glycerin in cattle feed: intake, digestibility, and ruminal and blood parameters

# Glicerina na alimentação de bovinos de corte: consumo, digestibilidade, parâmetros ruminais e sanguíneos

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## Abstract

This study aimed to evaluate the effects of glycerin supplements in the diet of beef cattle by assessing intake, apparent nutrient digestibility, ruminal pH, ruminal ammonia concentrations, and blood parameters. The study was conducted at the São Paulo State University (UNESP, Jaboticabal campus) using five crossbred cattle in an experiment employing a 5 x 5 Latin square design. Cattle diet treatments included zero, 50, 100, 150, and 200 g $kg^{-1}$ dry matter of glycerin. Feed, leftover feed, and faeces were collected to determine intake and digestibility. Samples of ruminal liquid were collected at –1, 0, 1, 2, 4, 6 and 8 h after feeding to determine pH and ruminal ammonia. Blood was collected four hours after the morning feeding from the coccygeal vein. Replacing maize with glycerin resulted in lower concentrations of ether extract and non-fibre carbohydrates in the diets, leading to a linear decrease in the intake of these nutrients ($P<0.05$). The digestibility of neutral detergent fibre and non-fibre carbohydrates also decreased linearly with increasing dietary glycerin concentrations ($P<0.05$). The results for ruminal fermentation parameters showed a linear decrease ($P<0.05$) in the ruminal concentration of $N\text{-}NH_3$ with increasing dietary levels of glycerin; however, ruminal pH was not affected ($P>0.05$). Serum concentrations of urea, triglycerides, cholesterol, and plasma glucose concentrations were within normal ranges based on the literature. The inclusion of glycerin in the cattle diet altered rumen fermentation, reducing the concentration of $N\text{-}NH_3$, the digestibility of neutral detergent fiber and non-fiber carbohydrates.

**Key words:** Beef cattle, biodiesel, glycerin, metabolism, ruminal fermentation

## Resumo

Objetivou-se com este trabalho avaliar os efeitos da inclusão de glicerina na dieta de bovinos de corte sobre o consumo, digestibilidade aparente dos nutrientes, pH ruminal, concentrações de amônia ruminal e parâmetros sanguíneos. O trabalho foi conduzido na Faculdade de Ciências Agrárias e Veterinárias-FCAV/Unesp, campus de Jaboticabal, utilizando-se cinco bovinos mestiços distribuídos em delineamento experimental quadrado latino 5 x 5. As dietas foram formuladas com a inclusão de 0, 50, 100, 150 e 200

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g $kg^{-1}$ de glicerina na MS. Para determinação do consumo e digestibilidade foram realizadas colheitas de alimentos, sobras e fezes. Para determinação do pH e amônia ruminal foram colhidas amostras de líquido ruminal nos tempos –1, 0, 1, 2, 4, 6 e 8 horas após a alimentação. As colheitas de sangue foram realizadas 4h após a alimentação matutina via veia coccígea. A substituição do milho pela glicerina resultou em menores concentrações de extrato etéreo e carboidratos não fibrosos nas dietas, o que levou a redução linear no consumo desses nutrientes (P<0,05). As digestibilidades da fibra em detergente neutro e de carboidratos não fibrosos apresentaram redução linear com o aumento da concentração de glicerina na dieta (P<0,05). Em relação à fermentação ruminal, houve decréscimo linear (P<0,05) na concentração ruminal de $N\text{-}NH_3$ com o aumento da inclusão desse subproduto, mas o pH ruminal não foi influenciado (P>0,05). As concentrações séricas de ureia, triglicerídeos e colesterol ficaram dentro dos níveis considerados normais pela literatura assim como as concentrações plasmáticas de glicose. A inclusão de glicerina na dieta de bovinos alterou a fermentação ruminal, reduzindo a concentração de $N\text{-}NH_3$, a digestibilidade da fibra em detergente neutro e dos carboidratos não fibrosos.
**Palavras-chave:** Biodiesel, fermentação ruminal, metabolismo, subproduto

## Introduction

Growing concern for the environment and the search for alternative sources of renewable energy has made biodiesel a subject of great interest and the centre of much attention from researchers (ABDALLA et al., 2008). In Brazil, biodiesel began to be added to diesel oil at concentrations of 50 g $kg^{-1}$ in January 2010 (ANP, 2012); however, the amount of glycerin generated by biodiesel production may jeopardise the positive ecological impacts of using biodiesel, given that glycerine corresponds to approximately 10% of the total volume of the biodiesel produced (GALLEGO et al., 2014).

From a nutritional standpoint, glycerin has emerged as a promising alternative energy source in animal feed, especially for cattle, because it is a gluconeogenic substance. There is a growing interest in using glycerin in animal feed, because biodiesel production has increased in recent years leading to increased stocks of glycerin resulting in low cost of this by-product (YAZDANI; GONZALES, 2007). Glycerin can be used as an alternative energy source in diets for cattle finished in feedlot, replacing conventional ingredients, such as maize. Many of the studies addressing the effects of using glycerin in cattle feed focus on animal performance and meat quality (MOORE et al., 2011; LEÃO et al., 2013; EIRAS et al., 2014; LAGE et al., 2014). Consequently, the effects of glycerin feed supplements on patterns of ruminal fermentation have not yet been fully elucidated.

This study aimed to evaluate the intake and apparent digestibility of dry matter, organic matter, crude protein, neutral detergent fibre, acid detergent fibre, ether extract, and non-fibre carbohydrates in addition to ruminal pH, ruminal concentrations of ammonia nitrogen, and blood parameters (glucose, cholesterol, triglycerides, and urea) in feedlot cattle supplied with diets containing increasing concentrations of glycerin.

## Material and Methods

The study was conducted at the School of Agricultural Sciences and Veterinary at São Paulo State University (UNESP, Jaboticabal campus) in accordance with ethical standards approved by the Ethics Committee on Animal Use of the UNESP-Jaboticabal (Protocol No. 022811/10).

### *Animals, diets and experimental design*

A total of five castrated crossbred cattle of approximately two years in age (initial weight = 500 ± 50 kg), fitted with permanent cannulas in the rumen, were used. The animals were housed in individual pens with free access to water containers and feed troughs. A rigorous sanitisation routine was maintained, and the pens and water containers were cleaned on a daily basis. Five experimental diets containing the same amount of protein (121.4 g $kg^{-1}$, DM basis) and metabolizable energy (2.6 Mcal

kg$^{-1}$, DM basis) were formulated according to NRC (1996) at a concentrate:roughage ratio of 60:40, with five different proportion of glycerin (zero, 50, 100, 150, and 200 g kg$^{-1}$ DM basis). The ingredients used in the diets included the following: maize silage, ground maize, soybean hulls, sunflower meal, salt, mineral supplement, and glycerin (Table 1). The glycerin was obtained from soybean oil and contained 830 g kg$^{-1}$ glycerol, 110 g kg$^{-1}$ water, 6 g kg$^{-1}$ salt (99 g kg$^{-1}$ NaCl), and 0.01 g kg$^{-1}$ of methanol.

**Table 1.** Ingredients and chemical composition of the experimental diets (dry mater basis).

| | Glycerin (g kg$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Item | 0 | 50 | 100 | 150 | 200 |
| Ingredients (g kg$^{-1}$ ) | | | | | |
| Maize silage | 400 | 400 | 400 | 400 | 400 |
| Ground maize | 392 | 330 | 273 | 219 | 156 |
| Soybean hulls | 73 | 81 | 85 | 87 | 79 |
| Sunflower meal | 125 | 129 | 132 | 134 | 155 |
| Mineral suplement[1] | 05 | 05 | 05 | 05 | 05 |
| Salt | 05 | 05 | 05 | 05 | 05 |
| Glycerin[2] | 0 | 50 | 100 | 150 | 200 |
| Chemical composition (g kg$^{-1}$) | | | | | |
| Crude protein | 124 | 123 | 121 | 120 | 119 |
| Neutral detergent fiber | 336 | 335 | 328 | 327 | 325 |
| Ether extract | 46 | 43 | 39 | 35 | 31 |
| Non-fibrous carbohydrate | 529 | 482 | 437 | 384 | 337 |
| Metabolizable energy (Mcal kg$^{-1}$) | 2.6 | 2.6 | 2.6 | 2.6 | 2.6 |

[1]Mineral Supplement for Cattle, warranty levels in 1000g: Phosphorus 40g, Calcium 80g, Sodium 195g, Chlorine 300g, Magnesium 5g, Sulfur 26g, Zinc 2000mg, Cupper 1000mg, Manganese 500mg, Cobalt 100mg, Iodine 100mg, Selenium 5mg, Fluor (max.) 400mg, vehicle q.s.p. 1000g; [2]Metabolizable energy = 3.2 Mcal/kg MS
**Source:** Elaboration of the authors.

The animals were fed twice a day at 08h00 and 16h00 and consumed clean water *ad libitum*. The treatments were randomly assigned to the animals in a 5 x 5 Latin square design experiment (five animals x five diets x five periods). The experiment lasted 125 days and consisted of five periods of 25 days, with the first 14 days being used for adaptation of animals to the diet, and the last 11 days being used for data collection.

*Sampling*

The feed was supplied at a rate such that leftover feed did not exceed 10% of the total supply, and the leftovers were collected and weighed daily. The feed was sampled at the beginning of each period. Samples of leftover feed were collected from the 15$^{th}$ to the 17$^{th}$ day of each period, totalling three samples/animal/period. Faecal samples (approximately 100 g) were collected from the 17$^{th}$ to the 19$^{th}$ day of the experimental period, directly from the floor after defecation at 2, 6, 10, 14, 18 and 22 h after the first feeding (08h00), totalling six samples/animal/period. The samples were taken from the upper surfaces of the stools to prevent contamination by the underlying soil.

After collection, the samples were dried in a forced-air circulation oven at a temperature of 55 ºC for 72 h and individually ground to one mm in a knife mill. At the end of each experimental period, a composite sample was made for each animal, based on the pre-dried weight of each sampling day. The

samples were analyzed for contents of dry matter (DM), mineral matter (MM), crude protein (CP), ether extract (EE) according to AOAC (1990) and acid detergent fiber (ADF) according to Van Soest, Robertson and Lewis (1991). To analyze the neutral detergent fiber (NDF), the samples were treated with thermostable alpha amylase without using sodium sulfite, corrected for residual ash (MERTENS, 2002) and for residual nitrogenous compounds (LICITRA; HERNANDEZ; VAN SOEST, 1996). Non-fibre carbohydrates (CNF) were calculated using the equation provided by Sniffen et al. (1992). The coefficients of apparent digestibility of DM and other nutrients were determined using indigestible acid detergent fibre (ADFi) as an internal indicator, which was determined using an *in situ* incubation technique with the supplied feed, feed leftovers, and faeces for 264 h according to Casali et al. (2008).

To determine pH and the concentration of ammonia in the rumen fluid, samples of rumen fluid (approximately 100 mL) were collected on the 21st day of each experimental period through a ruminal cannula one hour before the first feeding (-1), immediately before the first feeding (0) and at 1, 2, 4, 6, and 8 h after the first feeding (08h00). The pH of the samples was measured immediately after collection using a digital pH meter. Ammonia nitrogen concentration was determined by distillation of the sample with KOH (2 mol $L^{-1}$) in a micro-Kjeldhal apparatus followed by acid titration using HCl (0.005 mol $L^{-1}$). For the determination of the blood parameters, blood samples were collected with coccygeal venipuncture using Vacuotainer® tubes on the 25th day of each experimental period, four hours after the morning feeding. One blood sample (10 mL) was harvested without additives for cholesterol, triglycerides, and urea concentration analysis, whereas a second blood sample (5 mL) was harvested with 5 mg of sodium fluoride and 4 mg of potassium oxalate for subsequent glucose determination. All blood samples were centrifuged at 1,500 ×g at 4°C for 15 min. The plasma and serum analyses were performed using commercial kits.

*Statistical analysis*

The data were analysed using the MIXED procedure in Statistical Analysis System software (SAS 9.2, SAS Institute, Carry, NC, USA) according to the following statistical model:

$$Y = \mu + A_i + P_j + D_k + e_{ijkl},$$

where

$\mu$ = overall mean, $A_i$ = animal random effect ($i$ = 1 to 5), $P_j$ = period random effect ($j$ = 1 to 5), $D_k$ = diet fixed effect ($k$ = 1 to 5) and $e_{ijkl}$ = residual error.

For the data for ruminal pH and ruminal ammonia concentration, collection time was assumed to be a constant in the statistical model:

$$Y = \mu + A_i + P_j + D_k + T_l + D_k \times T_l + e_{ijkl},$$

where

$\mu$ = overall mean, $A_i$ = animal random effect ($i$ = 1 to 5), $P_j$ = period random effect ($j$ = 1 to 5), $D_k$ = diet fixed effect ($k$ = 1 to 5), $T_l$ = collection time fixed effect ($l$ = 1 to 7), $D_k \times T_l$ = i interaction between diet and collection time and $e_{ijkl}$ = residual error.

"Autoregressive" was the covariance structure that best fit the pH and ammonia data set. Orthogonal contrasts were performed to check for linear and quadratic effects of the inclusion of glycerin at a significance level of 0.05. All treatment means were calculated using the LSMEANS command.

## Results

*Intake and digestibility of nutrients*

The intake of DM, organic matter (OM), CP, NDF and ADF (kg $day^{-1}$) was not affected (P>0.05) by the diet treatments (Table 2) with mean values of 10.1; 8.7; 1.7; 3.3 and 2.1 kg $day^{-1}$, respectively. However, the intake of EE and NFC (kg $day^{-1}$) decreased linearly (P<0.05) with increasing dietary glycerin concentrations. EE intake decreased from 0.5 kg $day^{-1}$ for zero g $kg^{-1}$ of dietary glycerin to 0.3 kg $day^{-1}$ for 200 g $kg^{-1}$ of dietary glycerin, while NFC intake decreased from 5.2 kg $day^{-1}$ for zero g $kg^{-1}$ of dietary glycerin to 3.1 kg $day^{-1}$ for 200 g $kg^{-1}$ of dietary glycerin.

**Table 2.** Intake of dry matter and nutrients (kg/day) in cattle fed diets containing different proportions of glycerin (g $kg^{-1}$).

| Item | Glycerin (g $kg^{-1}$) | | | | | s.e.m. | Treatment P | Contrasts | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | | | L | Q |
| | | | | | | | | P | |
| Dry matter | 10.2 | 10.4 | 10.4 | 10.0 | 9.3 | 0.33 | 0.285 | 0.101 | 0.138 |
| Organic matter | 8.8 | 8.9 | 9.0 | 8.6 | 8.1 | 0.28 | 0.287 | 0.101 | 0.141 |
| Crude protein | 1.3 | 1.3 | 1.3 | 1.2 | 1.2 | 0.04 | 0.224 | 0.082 | 0.578 |
| NDF | 3.4 | 3.5 | 3.4 | 3.3 | 3.0 | 0.11 | 0.193 | 0.061 | 0.186 |
| ADF | 2.1 | 2.2 | 2.1 | 2.1 | 2.0 | 0.06 | 0.308 | 0.100 | 0.164 |
| Ether extract | 0.5 | 0.5 | 0.4 | 0.4 | 0.3 | 0.02 | <0.001 | <.0001 | 0.172 |
| NFC | 5.2 | 4.9 | 4.5 | 3.8 | 3.1 | 0.21 | <0.001 | <.0001 | 0.115 |

NDF= neutral detergent fiber; ADF= acid detergent fiber; NFC = Non-fibrous carbohydrates; Orthogonal contrasts: L = linear effect of glycerin proportion, Q = quadratic effect of glycerin proportion.
**Source:** Elaboration of the authors.

The apparent digestibility of DM, OM, CP, ADF and EE was not affected (P>0.05) by the treatments with mean values of 61.2, 62.7, 68.5, 38.5, and 86.3%, respectively (Table 3). A linear decrease in digestibility was observed for the NDF and NFC fractions with increasing glycerin (P<0.05) with a decrease from the zero g $kg^{-1}$ to the 200 g $kg^{-1}$ glycerin treatment of 28.8 and 6.1% for NDF and NFC, respectively.

**Table 3.** Digestibility of dry matter and nutrients (%) in cattle fed diets containing different proportions of glycerin (g $kg^{-1}$).

| Item | Glycerin (g $kg^{-1}$) | | | | | s.e.m. | Treatment P | Contrasts | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | | | L | Q |
| | | | | | | | | P | |
| Dry matter | 62.7 | 65.6 | 61.4 | 58.8 | 57.6 | 1.05 | 0.187 | 0.055 | 0.469 |
| Organic matter | 64.1 | 66.7 | 62.9 | 60.2 | 59.6 | 1.01 | 0.263 | 0.062 | 0.556 |
| Crude protein | 70.5 | 73.4 | 68.9 | 67.8 | 61.9 | 1.96 | 0.567 | 0.162 | 0.450 |
| NDF | 46.8 | 52.4 | 41.5 | 34.9 | 33.3 | 2.41 | 0.027 | 0.004 | 0.407 |
| ADF | 42.6 | 45.1 | 39.1 | 34.2 | 31.5 | 2.31 | 0.370 | 0.077 | 0.833 |
| Ether extract | 89.1 | 87.6 | 86.9 | 84.1 | 83.8 | 0.77 | 0.087 | 0.068 | 0.944 |
| NFC | 82.0 | 82.2 | 80.7 | 80.3 | 77.0 | 0.67 | 0.015 | 0.002 | 0.121 |

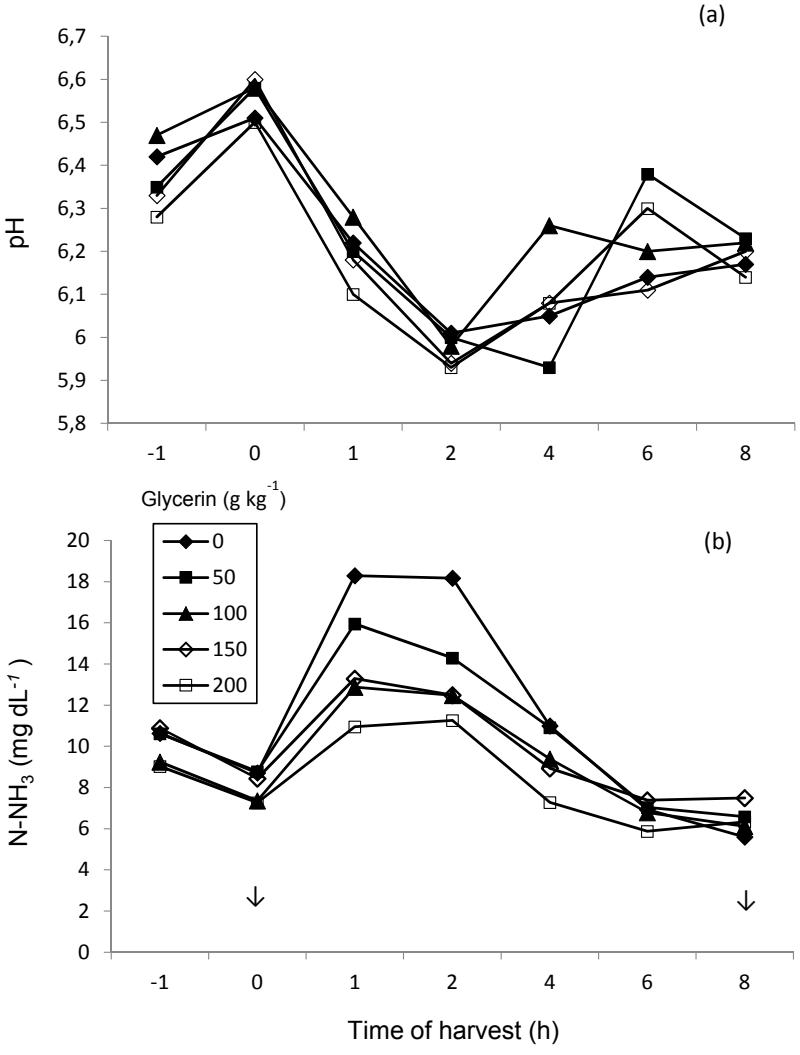
NDF= neutral detergent fiber; ADF= acid detergent fiber; NFC = Non-fibrous carbohydrates; Orthogonal contrasts: L = linear effect of glycerin proportion, Q = quadratic effect of glycerin proportion.
**Source:** Elaboration of the authors.

*Ruminal pH and ammonia nitrogen concentration*

A time x treatment interaction effect was not detected for pH, nor was ruminal concentration of $N\text{-}NH_3$ detected (P>0.05). Therefore, only the main effects from the model are discussed in this report. Ruminal pH was not affected by level of glycerin in the diet (P>0.05) and had a mean value of 6.2 (Figure 1a). The lowest pH values occurred between two and four hours after the feeding (5.9 and 6.1, respectively). The addition of glycerin to the diet resulted in a linear decrease in the ruminal concentration of ammonia (P<0.05). Ammonia concentrations for the zero glycerin treatment were 26.5% higher on average than the concentrations

for the 200 g $kg^{-1}$ of glycerin treatment (Figure 1b). The maximum values of N-$NH_3$ occurred between one and two hours after feeding, with a decrease at four hours after feeding. The highest concentrations of N-$NH_3$ were 18.3, 15.9, 12.9, 13.3, and 11.3 mg N-$NH_3$ $dL^{-1}$ for the zero, 50, 100, 150, and 200 g $kg^{-1}$ of glycerin treatments, respectively.

**Figure 1** Ruminal pH (a) and ruminal ammonia concentration (b) of cattle fed diets containing different proportion of glycerin according to the time of harvest. Arrows indicate the time of feeding. Orthogonal contrasts (effect of glycerin proportion): P-value (a): Treatment, P = 0.596; Linear, P = 0.5563; Quadratic, P = 0.2395; s.e.m. = 0.021. P-value (b): Treatment, P = 0.013; Linear, P = 0.0198; Quadratic, P = 0.8268; s.e.m. = 0.300.




**Source:** Elaboration of the authors.

*Blood parameters*

The treatments did not affect blood concentrations of triglycerides, cholesterol and urea (P>0.05; Table 4), which had mean values of 7.29, 108.6 and 21.45 mg $dL^{-1}$, respectively, corresponding to normal values according to González and Silva (2006), normal values are 7-14, 80-180 and 17-45 mg $dL^{-1}$ for triglycerides, cholesterol and urea, respectively.

The treatments had a quadratic effect on glucose concentration (P<0.05), with glucose ranging from 50.91 (minimum value) in the zero g $kg^{-1}$ glycerin treatment to 73.09 mg $dL^{-1}$ (maximum value) in the 100 g $kg^{-1}$ of glycerin treatment.

**Table 4.** Plasma glucose and serum cholesterol, triglycerides and urea concentrations (mg $dL^{-1}$) in cattle fed diets containing different proportions of glycerin (g $kg^{-1}$).

| Item | Glycerin (g $kg^{-1}$) | | | | | s.e.m. | Treatment P | Contrasts | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | | | L | Q |
| | | | | | | | | P | |
| Glucose | 50.9 | 67.5 | 73.0 | 60.7 | 52.4 | 2.82 | 0.001 | 0.713 | 0.0001 |
| Cholesterol | 141.1 | 88.6 | 103.2 | 113.3 | 96.7 | 7.28 | 0.089 | 0.133 | 0.179 |
| Triglycerides | 8.9 | 7.3 | 8.2 | 5.3 | 6.8 | 1.32 | 0.977 | 0.854 | 0.917 |
| Urea | 21.5 | 21.2 | 21.5 | 23.1 | 19.9 | 1.40 | 0.896 | 0.846 | 0.605 |

Orthogonal contrasts: L = linear effect of glycerin proportion, Q = quadratic effect of glycerin proportion.
**Source:** Elaboration of the authors.

## Discussion

*Intake and digestibility of nutrients*

The increased glycerin concentrations in the cattle diets did not reduce dry matter intake. The literature shows disagreement regarding dry matter intake, which may be affected by the composition of the glycerin and the presence of such substances as salts and methanol, which vary according to the biodiesel production process (CHUNG et al., 2007). Furthermore, the purity of the glycerin may also influence its acceptability to the animals. Several previous studies have shown no reduction in dry matter intake for animals fed glycerin (CARVALHO et al., 2011; FARIAS et al., 2012; BARTON et al., 2013). Van Cleef et al. (2014), used glycerin with a similar composition to the glycerin used in this study (obtained from biodiesel production from soybeans, comprised of 860 g $kg^{-1}$ glycerol, 950 g $kg^{-1}$ water, 60 g $kg^{-1}$ salts, and less than 0.1 g $kg^{-1}$ methanol) and found no difference in dry matter intake for feedlot Nelore bulls fed up to 300 g $kg^{-1}$ glycerin by dry weight.

Nevertheless, other studies reported a reduction in dry matter intake in cattle that were fed glycerin (PYATT; DOANE; CECAVA, 2007; ELAM et al., 2008; PARSONS; SHELOR; DROUILLARD, 2009). The literature suggests numerous possible causes, ranging from the effects of glycerin on ruminal metabolism to its effects on intermediary metabolism. Roger et al. (1992) observed that a dietary addition of 50 g $kg^{-1}$ glycerol inhibited the growth and cellulolytic activity of rumen bacteria and that dietary concentrations above this value could alter ruminal fermentation and result in reduced dry matter intake. Moreover, several studies have determined that even when a reduction in dry matter intake occurs with increased dietary glycerin concentrations, weight gain and feed efficiency are not affected (PYATT; DOANE; CECAVA, 2007; PARSONS; SHELOR; DROUILLARD, 2009).

The reduction in EE and NFC intake observed in this study was expected, given that because glycerin does not contain significant amounts of EE or carbohydrates, there were lower concentrations of these nutrients in the diets that contained glycerin. Table 1 shows that substituting glycerin for maize reduced dietary EE concentrations from 46 to 31 g $kg^{-1}$ for the zero and 200 g $kg^{-1}$ glycerin treatments, respectively. Maize was also the main quantitative source of NFC in the diet. When the maize was replaced by glycerin, a decrease in the

concentration of NFC in the diet was observed, resulting in a decrease in NFC intake as dietary glycerin concentrations increased.

Neutral detergent fiber and NFC digestibility decreased linearly as the amount of glycerin in the diet increased. The impact of glycerin on fiber digestion has been studied by several research groups, and studies have indicated that the inclusion of glycerin in cattle diets causes decreases in the use of the fibre fraction by the animals (PARSONS; SHELOR; DROUILLARD, 2009, VAN CLEEF et al., 2014). The digestion of fibre is of particular interest in studies of glycerin diets because the inhibitory effects of glycerin on cellulolytic bacteria and fungi activity are clearly evident and provide a plausible explanation for reduced fibre digestion, which has been observed *in vitro* and *in vivo* (DROUILLARD, 2012). Similarly, Roger et al. (1992) observed reductions in growth and cellulose degradation by cellulolytic bacteria and fungi *in vitro* in media containing five and 50 g $kg^{-1}$ of glycerin, while Abo El-Nor et al. (2010) observed reductions in NDF digestibility and DNA concentrations of *Butyrivibrio fibrosolvens* and *Selenomonas ruminantium* bacteria with increased glycerin concentrations in the diet of Holstein cows. *B. fibrosolvens* bacteria are involved in cellulose and hemicellulose degradation, and *S. ruminantium* bacteria are involved in the degradation of starch and soluble sugars in the rumen (ARCURI; LOPES; CARNEIRO, 2011). The mechanism of action of glycerin on the populations of fibrolytic bacteria, and consequently on digestibility and of fibrous fraction of diets, is still unclear. It may be related with formation of an environment unfavorable to the multiplication of these bacteria or physical protection of fibrous particles preventing the adhesion of bacteria (VAN CLEEF et al., 2014).

*Ruminal pH and ammonia nitrogen concentration*

There were no observed effects of the treatments on ruminal pH. The lowest pH values occurred between two and four hours after feeding (5.9 and 6.1, respectively). According to Valadares Filho and Pina (2011), ruminal pH can range from 5.5 to 7.2, with the lowest values being observed shortly after feeding the animals with high-concentrate diets; while pH values below 6.0 may inhibit the activity of bacteria that perform cellulose fermentation. The pH values in this study were not sufficiently low to affect ruminal fermentation because pH values below 6.0 were observed only at two hours after feeding, and the pH did not remain at suboptimal levels for an extended period of time. Similarly, Abo El-Nor et al. (2010) did not find an effect of glycerin on ruminal pH in Holstein cows fed diets with glycerin concentrations of up to 108 g $kg^{-1}$.

Decreases in pH result from intense ruminal fermentation and consequent increases in the production of short-chain fatty acids and lactate (DONKIN, 2008). The inclusion of glycerin in the diet can affect the rate of digestion and the formation of end products, depending on the amount of glycerin. In the rumen, the glycerol is mainly fermented into propionate (FERRARO et al., 2009; LEE et al., 2011), but several studies show that glycerol may be absorbed directly through the rumen wall (KREHBIEL, 2008). According to Trabue et al. (2007), glycerol quickly disappeared in rumen, *in vitro* tests showed that over 80% of glycerol disappeared within 24 hours. Thus, increases in the concentration of glycerin in the diet did not promote the production of products, such as lactate, that can cause a decrease in ruminal pH. These observations indicate that the addition of glycerin to cattle diets in this study affected ruminal microorganism populations because reduced fibre digestibility was even observed when ruminal pH remained at appropriate values for fermentation of the fibre fraction. Therefore, the reduction in NDF digestibility may be related to changes in the population of bacteria responsible for the degradation of this fraction that resulted from the presence of glycerin rather than from changes in pH.

Increased glycerin in the diet was correlated with a linear decrease in N-$NH_3$ concentrations. Wang et al. (2009) supplied Simmental cattle with zero, 100, 200, and 300 g of dietary glycerin/animal/day and observed a similar linear decrease in N-$NH_3$ concentrations. For all of the treatments, the N-$NH_3$ concentration was higher than 10 mg $dL^{-1}$ (LENG, 1990), which is considered the minimum value for appropriate ruminal fermentation. However, it is worth noting that 10 mg $dL^{-1}$ is the minimum concentration of N-$NH_3$ required to maintain microbial synthesis, whereas the ideal value of N-$NH_3$ for fermentation, especially of fibre, is between 15 and 29 mg $dL^{-1}$ (PRESTON, 1986). In this study, the only values of N-$NH_3$ concentration within this ideal range occurred at one-two hours after feeding in the zero and 50 g $kg^{-1}$ glycerin treatment groups. The reduction in ammonia nitrogen may also have contributed to the decrease in NDF digestibility because cellulolytic bacteria require ammonia for growth. Thus, the observed decrease in N-$NH_3$, especially in the treatments with a glycerin concentration greater than 50 g $kg^{-1}$, may have negatively affected the digestion of fibre.

*Blood parameters*

Urea is a key indicator of animal protein intake and diet is the primary factor affecting serum urea levels. According to Wittwer (2000), reduced energy ingestion is an inverse response to increased concentrations of ruminal ammonia. With the high availability of energy from glycerin and the decrease in ruminal N-$NH_3$ concentrations observed in this study, a decrease in plasma urea concentrations was expected but not observed. Similarly, serum triglyceride concentrations were not affected by the treatments, and the triglyceride values fell within the range considered to be ideal (7-14 mg $dL^{-1}$) according to González and Silva (2006). An increase in serum triglyceride concentrations may cause hepatic lipidosis. This type of pathology is most evident in animals that have a high level of concentrate in their diet (over 70%) for long periods of time. In this study the animals received a diet that was 60% concentrate, which may have helped maintain normal levels of triglycerides in the blood.

Energetic metabolism can be estimated by blood glucose and cholesterol (FERNANDES et al., 2012). The diet treatments had quadratic effects on glucose and there were no effects on cholesterol concentrations. A large portion of the glycerol fermented in the rumen is transformed into propionic acid (LEE et al., 2011), which is the precursor of gluconeogenesis in ruminants. The hypothesis for the quadratic effects is that, there was higher glycerol fermentation in 100 g $kg^{-1}$ than in 200 g $kg^{-1}$, from which a larger portion escapes ruminal fermentation and it was absorbed intact (WILBERT et al., 2013) decreasing de blood glucose. It is important to note that even with the observed effect of diet on blood glucose and cholesterol concentrations, all of the values obtained for these two parameters were within normal ranges (45-75 mg $dL^{-1}$ and 80-120 mg $L^{-1}$ for glucose and cholesterol, respectively) (GONZÁLEZ; SILVA, 2006).

## Conclusions

The inclusion of up to 200 g $kg^{-1}$ of glycerin in cattle diets does not affect dry matter intake nor alter the blood parameters of the animals. Further studies are needed to understand the process of glycerin metabolism that leads to decreases in the concentration of ammonia nitrogen in the rumen and the digestibility of fibre and non-fibre carbohydrates but does not affect pH.

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