Replacement of corn by crude glycerin in beef cattle diets: ruminal fermentation, rumen kinetic and blood metabolites

Sustitución del maíz por glicerina cruda en dietas para ganado de carne: fermentación, cinética ruminal y metabolitos sanguíneos

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ABSTRACT

With the expansion of the biodiesel industry, there is a potential in the availability of processing byproducts, especially crude glycerin (CG), which has high energy content and can be used in animal feeding. The objective of this study was to evaluate the effects of CG supplementation on fermentation, kinetic rumen and blood metabolites. 5 ruminally cannulated Nelore steers (522±43 Kg) were used in a 5×5 Latin square design. The treatments were: (control), 3, 6, 9 and 12% of CG based on the total dry matter. The estimated minimum point (pH min), average pH and average NH_3-N decreased linearly (P<0,05) as the level of CG in diet increased. Acetate decreased linearly as the level of CG in the diet increased. The propionate and butyrate increased linearly as the level of CG in diet increased (P<0,05). The acetate: propionate ratio decreased with the level of CG in diets. The rate of liquid passage (Kp), the ruminal volume (RV), the retention time (RT) and the recycling rate (Rec R) were not influenced by the levels of CG in the diet. There were no differences (P>0,05) between treatments for glucose, cholesterol, triglycerides and plasma urea nitrogen values (PUN). It is concluded that CG can be used to feed cattle at a level of 12% and can be considered as an alternative energy source.

Key words: biodiesel, byproduct, glycerol, ruminants

RESUMEN

Con la gran expansión de la industria del biodiesel, existe una disponibilidad potencial de los subproductos proveniente de su procesamiento, especialmente de la glicerina cruda (GC), la cual tiene un alto contenido energético y puede ser utilizada en la alimentación animal. El objetivo de este estudio fue evaluar los efectos de la suplementación con GC, sobre la fermentación, cinética ruminal y metabolitos sanguíneos. 5 novillos Nelore (522±43 Kg) canulados en el rumen, fueron distribuidos en un diseño de cuadrado latino 5X5. Los tratamientos fueron: (control), 3, 6, 9 y 12% de GC en base a la materia seca total de la dieta. El pH min, pH promedio y el $N-NH_3$ promedio disminuyeron (P<0,05) con el aumento de los niveles de GC en la dieta. El acetato disminuyo con el aumento de los niveles de GC en la dieta. Las concentraciones de propionato y butirato aumentaron (P<0,05) con los niveles de GC en la dieta. La proporción acetato: propionato disminuyó con el aumento de los niveles de GC en la dieta. La tasa de pasaje de líquidos (Kp), el volumen ruminal (VR), el tiempo de retención (TR) y la tasa de reciclaje (T Rec) no fueron influenciados por los niveles de GC en las dietas. No hubo diferencias (P>0,05) entre tratamientos para los niveles de glucosa, colesterol, triglicéridos y nitrógeno ureico en plasma. Se concluye que la GC puede ser usada en dietas de ganado de carne hasta en un nivel de 12%, además puede ser considerada como una fuente alternativa de energía.

Palabras clave: biodiesel, subproducto, glicerol, rumiantes.

INTRODUCTION

In Brazil, the production of biodiesel is significantly accelerated, because the government established mandatory adding 5% of biodiesel to petroleum diesel since 2013, according to Law 11097/2005 about the biodiesel introduction into Brazilian energetic matrix. With the great expansion of the biodiesel industry, there is a potential in the availability of processing co-products, especially CG, which has high energy content and can be used in animal feeding. The high offer of CG from biodiesel fuel production will likely flood glycerin supplies for the traditional uses, although there are many applications for glycerin, such as using it as an energy source in livestock diets (Chanjula *et al.,* 2014). The cattle production system in feedlots in Brazil has undergone significant changes in recent years, especially in the size of enterprises, management and animal nutrition.

This system is characterized by using high amounts of corn. The trend in grain prices is increasing, besides being designed for ethanol production, mainly in the United States, thus, the use of corn in the diet of ruminants is becoming limited. In this regard, the use of CG has emerged as an option for animal feeding due to lower cost and the ability to replace energy food, like corn (Mach *et al.,* 2008).

There is a great interest in the use of alternative feeds that can replace some of the grains used in the concentrates, without damaging the physiological, metabolical aspects or animal performance, due to this fact, the cost of confined animal production is considered very high (Benedeti *et al.,* 2016). However, it is noteworthy that the CG obtained from the process of trans-esterification of vegetable oil in Brazil has not been standardized related to composition, although in recent years many efforts and new technologies have been studied to obtain a co-product of better quality.

Preliminary studies on the inclusion of CG in the feeding of ruminants, from the earliest to the latest (Johns, 1953; Avila *et al.,* 2011), indicate that glycerol is fermented to propionate. This is of a great interest in the beef cattle nutrition and has been tested by Chanjula *et al.* (2015) who observed that most of the glycerol has become propionic acid. According to Trabue *et al.* (2007),

when CG is provided in animal diet it tends to reduce the available amount of carbon and ruminal hydrogen contributing to the production of methane gas, by reducing the production of acetate, with subsequent improvement in the efficiency of energy utilization by the animal.

Furthermore, Elam *et al.* (2008) suggested that the presence of glycerol in the diet can increase the water holding capacity of the portions in low humidity environments and improve the palatability of the concentrate due to its flavor and mild taste. However, all these advantages need to be evaluated in *in vivo* experiments with the objective of building a scientific basis by nutritionists. This work was performed in order to evaluate the effects of adding CG into diets offered to beef cattle on fermentation, ruminal kinetics and blood metabolites.

MATERIAL AND METHODS

The experiment was performed in the Iguatemi Experimental Farm and the Feed and Animal Nutrition Analysis Laboratory, in the Universidade Estadual de Maringá, Brazil. 5 Nelore steers with 565±45 kg body-weight and a rumen cannula were housed in individual pens. The diets were formulated to allow 1,10 to 1,20 Kg d^{-1} daily weight gain. A 5x5 Latin square design was used with, 5 periods and 5 steers per treatment. The experimental periods were of 21d each with the first 15d intended for adaptation to the experimental diets (Table 1) and the following 6d for sample collection (feeds offered, rejections, rumen liquid, and blood plasma). Food intake was adjusted to obtain 5-10% of rejections from the total food offered. Daily food intake was calculated as the difference between food supplied and food rejected, based on the dry matter (DM).

The chemical composition of materials used in the experimental diets is shown in Table1 (ratio between forage and concentrate was 40:60). The CG inclusion in feedlot diets consisted of 0, 3, 6, 9 and 12% based on the total DM of the diet. The diets were provided *ad libitum* a complete mixture twice a day (at 08:00 h and at 16:00 h), and the CG was mixed completely with the other ingredients during the feed preparation.

During the 21d, samples were collected using a ruminal cannula, 150ml of ruminal fluid to

Table 1. Chemical and feed raw material composition of experimental diets based on DM.

	Experimental diets composition (%)				
	0	3	6	9	12
Sorghum silage	40,0	40,0	40,0	40,0	40,0
Corn	30,2	26,4	23,2	19,7	16,2
Soybean meal	8,0	8,8	9,0	9,5	10,0
Wheat flour	20,0	20,0	20,0	20,0	20,0
Crude glycerin	0,0	3,0	6,0	9,0	12,0
Calcium carbonate	1,0	1,0	1,0	1,0	1,0
Sodium chloride	0,3	0,3	0,3	0,3	0,3
Mineral premix[1]	0,5	0,5	0,5	0,5	0,5
	Chemical composition (%)				
Crude Protein	13,5	13,5	13,5	13,5	13,5
Ether Extract	3,1	3,2	3,2	3,2	3,2
Neutral Detergent Fiber	36,7	36,4	36,1	35,8	35,5
Non Fiber Carbohidrates[2]	43,1	43,2	43,6	43,9	44,2
Total DigestibleNutrients[2]	71,0	71,0	71,0	71,0	71,0
NEm[3]	1,67	1,67	1,67	1,67	1,67
NEg[4]	1,06	1,06	1,06	1,06	1,06

[1]Mineral premix: 0,023% calcium iodate; 0,127% zinc oxide; 0,0089% sodium selenite; 0,03 % cobalt sulfate; 1,2% copper sulfate; 2,07% manganese sulfate, [2]Calculated according Sniffen *et al.* (1992); [3]NEm= net energy for maintenance and [4]NEg= net energy for gain, calculated by NRC (2000).

determine pH, concentration of ammonia nitrogen (NH_3-N) and ruminal kinetics of liquid phase by the cobalt complex ethylenediamine tetra acetic (EDTA-Co). The first collection was started immediately before the delivery of the first meal of the day, which was collected at time 0 and the next sample at 2, 4, 6, 8, 12, 14 and 24h after the first feeding. After each ruminal fluid collection, the pH was immediately measured with a digital pH meter (Digimed DM20).

For the analysis of ammonia nitrogen (NH_3-N), an aliquot of 50ml of ruminal fluid was acidified with 1ml of H_2SO_4 (1:1) and stored at-20°C for later analysis. The rumen fluid was kept at room temperature and then it was centrifuged at 3.000xg for 15 min. The NH_3-N concentration of the samples was determined using the Fenner (1965) method.

To determine the kinetics of liquid phase, 30g of Co-EDTA diluted in 500ml of distilled water were administered into the rumen of each animal, before the first feeding and placed directly into the rumen at different locations in a single dose for to determine the liquid passage rate (Udén *et al.,* 1980). Similarly, 50ml of rumen fluid were collected approximately before placing the Co-EDTA and every 2 hours to complete 12 hours, with a final collection 24 hours after administration of the tracer.

The liquid passage rate and ruminal concentration curves of Co-EDTA were adjusted to an exponential model unicompartmental of Hungate (1966):

$Yco = A.e^{(-k1.t)}$.

Where, Y_{CO} = tracer concentration at time t;

A = equilibrium concentration of cobalt;

K1 = rate of passage or dilution of cobalt;

t = sampling time.

The dynamic parameters of the liquid phase were calculated according to the methodology of Colucci et al. (1990) where: the retention time in the rumen (hours)= 1/fluid passage rate (TpRet=1/kl%/h); ruminal volume (liters) provided the quantity of cobalt (mg)/A mg/L (VR=Co/A); recycling rate of ruminal liquid phase (number of times/day) =24h/TpRet, calculated according Maeng and Baldwin, (1976).

To determinate the volatile fatty acid (VFA) samples were collected at time 0 and the next sample at 2, 4, 6, 8, 12 hours, after the first feeding. Samples of 50 ml of ruminal fluid were collected and freezed until analysis. Samples were centrifuged at 15.000xg for 20 min and the supernatant was used to determine VFA concentration by gas chromatograph (Hewlett Packard 5890 Series II) equipped with integrator (Hewlett Packard 3396 Series II Integrator) and automatic gun (Hewlett Packard 6890 Series Injector), and analyzed according to Campos et al. (2004).

The internal standard used was 2-methylbutyric acid being added in each tube for reading chromatograph, 100 µl of internal standard, 800 µl and 200 µl sample of formic acid. A short chain mixture of fatty acids with a known concentration was used as an external standard for integrator calibration.

Blood samples were collected on the 18th day of each period, before the first feeding (8h00min) by jugular vein puncture using heparin as anticoagulant. Subsequently, the samples were centrifuged for 15min at 2500xg, the plasma transferred to micro tubes considering pet / time / period. The plasma resultant was stored at -20°C for subsequent analysis of glucose, cholesterol, triglycerides, and plasma urea nitrogen using commercial kits (GoldAnalisa®).

The experimental design was a 5x5 Latin square. The data was interpreted using ANOVA, adopted a 5% probability, and when there was a significant treatment effect it was used for a polynomial regression analysis.

Data of pH and NH_3-N in the ruminal fluid were adjusted using a time function equation for each animal within each treatment period. These equations calculated the time to reach maximum acidity (pH), maximum and minimum concentrations of NH_3-N, using regression analysis. The critical points as biological variables were analyzed by ANOVA and when the values were significant, polynomial regression equations were analyzed in function of the increasing levels of CG. All statistical procedures were performed using SAS software (version 9.1 Statistical Analysis System, 2005).

RESULTS AND DISCUSSION

The dry matter intake (DMI), expressed as Kg/d were similar among treatments (P>0,05). The DMI ranged from 9,53 to 10,11 Kg/d and occurred in treatments with 9% and 3% of GC inclusion, respectively (Table 2).

Mach et al. (2008) reported that the existing methanol in CG can have potentially negative effect on the DMI, but the presence of 0,09% of this compound did not affect the intake of animals; and concluded that CG may be included as an alternative energy source to replace grain up to the level of 12%. In the present study 1,5% methanol in CG did not result in significant changes on DMI. Indeed, Lage et al. (2010) reported that ruminants do not have health risks associated with methanol in CG inclusion in the diet because methanol is naturally produced in the rumen as a result of pectin fermentation.

The minimum critical point (CP min) for pH was not influenced (P>0,05) by CG levels. However, the average pH and pH min decreased as the level of CG in the diet increased (P<0,05). The pH min changed from 6,51 to 6,29 for the treatments of 0% and 12% of CG, respectively. While the average pH fell from 6,70 to 6,49 for treatments of 0% and 12% of CG, respectively.

Wang et al. (2009) reported similar behavior of steers fed with inclusions of 0,100, 200 and 300 g/d of CG, observed values of ruminal pH of 6,58; 6,56; 6,32 and 6,23 respectively. Similar

Table 2. Dry matter intake (DMI) and critical point (hours: minutes) for pH and NH_3-N

Item	Levels of crude glycerin in the diet (%)					SE	P
	0	3	6	9	12		
DMI (kg/d)	9,64	10,11	9,23	9,53	9,86	0,21	0,35
CP mín[1] pH	4:57	4:41	4:41	4:40	4:25	0,19	0,45
pH in CP min*	6,51	6,46	6,40	6,29	6,23	0,05	0,01
pH average [3]*	6,70	6,63	6,67	6,53	6,49	0,03	0,01
CP max[2] NH_3-N	1:23	1:39	1:39	1:51	1:37	0,16	0,41
CP min NH_3-N	6:28	6:47	6:15	6:25	6:04	0,23	0,31
NH_3-N in CP min*	5,00	5,74	6,21	6,85	6,78	0,76	0,01
NH_3-N in CP max	17,07	17,40	15,91	13,54	10,33	1,07	0,43
NH_3-N average[3]*	10,94	11,43	11,21	10,17	8,51	0,72	0,03

[1]CP min= Minimum critical point during the first 8 hours post feeding, [2]CP max = Maximum Critical point during the first 8 hours post feeding. [3]Average value during the period from 0 to 8 hours post feeding. SE = standard error of the mean. P = Value P. *Regression equations: pH in CP min: $\hat{Y}$= 6,61-0,07X; pH average: $\hat{Y}$ =6,73-0,05X; NH_3-N in CP max: $\hat{Y}$ =20,05-1,73X and NH_3-N average: $\hat{Y}$ =12,29-0,61X.

results were reported by Defrain *et al.* (2004) who observed a pH decreased with the addition of CG (80,2% glycerol) in the diet of dairy cows postpartum; this researchers obtained 6,9; 6,89 and 6,61 values for 0; 430 and 860g / day levels of CG, respectively.

Despite the drop in pH in the treatments with higher levels of CG, the values are still higher than considered minimum desirable of 6,2 as reported by Hoover (1986), Ørskov (1988) and Van Soest (1994) to be ideal to promote fermentation of fiber and hold the activity of cellulolytic microorganisms.

The maximum critical point (CP max) and the CP min for the NH_3-N were not affected by the inclusion of CG (P>0,05), and the observed values occurred from 1:23 - 1:51 (hours: minutes) to CP max and from 6:04 - 6:47 (hours: minutes) to CP min post feeding. The minimum concentration of NH_3-N was not influenced by the levels of CG in the diet and it was found that for all treatments the values remained above 5 mg/dL[-1] as suggested by Satter and Slyter (1974) at least for an adequate ruminal fermentation of the cell wall. In respect to NH_3-N in CP max and average NH_3-N it was observed that as the level

of CG increases, the concentration of NH_3-N decreases linearly (P<0,05) possibly due to the presence of glycerol in the diet, leading to high growth of microbial populations in the rumen, increasing the consumption of NH_3-N, especially for populations that degraded the fiber. Similar results were observed by Wang *et al.* (2009) in steers fed with diets 60% forage and 40% concentrate, with concentrations of 10,4; 9,3; 7,9 and 7,5 mg/100 ml to levels of 0, 100, 200 and 300g /d of CG respectively.

Another reason for this behavior can be reduced proteolytic activity. This theory is supported by Paggi *et al.* (1999) who reported a decrease in proteolytic activity of 20% when glycerol was included at levels up to 300 mm in *in vitro* experiment. The same authors reported that when the glycerol is dissolved in the rumen, proteolysis is more difficult due to the lack of a hydrophobic chain of the glycerol molecule.

The VFA concentrations vary (P<0,05) according to the level of inclusion of CG in diet (Table 3). Acetate decreased linearly from 55,9 to 46,8 mmol/L as increased the level of CG in the diet. At the same time, the propionate increased linearly as the level of CG increased in diet (P=0,0005).

Table 3. Volatile fatty acids (VFA) depending on crude glycerin levels in beef cattle diets.

Item	Levels of crude glycerin in diet (%)					SE	P
	0	3	6	9	12		
Acetic Acid mmol/L*	55,9	56,7	50,4	48,8	46,8	5,12	0,0005
Propionic acid mmol/L*	11,7	14,7	16,4	19,1	19,2	3,88	0,0050
Butyric acid mmol/L*	11,7	13,1	15,3	18,0	18,5	6,39	0,0001
VFAs Total mmol/L	80,5	84,6	82,1	85,9	84,5	4,49	0,3600
ratio acetic: propionic*	4,5	3,9	3,1	2,7	2,6	0,57	0,0001

*Regression equations: acetic acid: $Y= 59,56 – 2,61x$ $r^2= 0,41$; Propionic acid: $Y= 11,34 + 1,70(x)$ $r^2= 0,29$; Butyric acid: $Y= 9,76 + 1,86(x)$ $r^2= 0,61$; Ratio acetic:propionic: $Y= 4,82 - 0,49(x)$ $R^2= 0,54$. SE = standard error of the mean. P = value P.

Similar behavior was observed with butyrate which increased from 11,7 in the diet with 0% to 18,5 mmol/L in the diet with 12% CG. These results directly influenced the acetate: propionate ratio which went from 4,5 in the treatment with 0% to 2,6 in the treatment with 12% CG.

Previous studies have shown that when animals (Defrain *et al.,* 2004; Trabue *et al.,* 2007) or cultures (Ferraro *et al.,* 2009; AbuGhazaleh *et al.,* 2011; Avila *et al.,* 2011; Chanjula *et al.*, 2015) were supplemented with glycerol, the acetate level was lower than in those without (glycerol). The results observed in this study are consistent with those reported by AbuGhazaleh *et al.* (2011), who replaced corn with glycerol with 99,5% purity at levels of 0, 15, 30 and 45% using continuous culture fermenters, similar behavior was also observed in the production of acetate, propionate and butyrate. Roger *et al.* (1992) and Paggi *et al.* (1999) reported that the cellulolytic activity of ruminal extract was reduced as glycerol concentration in diets increased. However, the reduction in fiber digestibility is usually associated with a decrease in acetate production (Castillejos *et al.,* 2006).

The propionate increase is consistent with other studies that have shown that glycerol fermentation resulted in the increases of propionate (Bergner *et al.,* 1994; Ferraro *et al.,* 2009; AbuGhazaleh *et al.,* 2011). Defrain *et al.* (2004) also observed that with a dose of 430 g/day glycerol, acetate tend to be reduced (P=0,15), but propionate was increased (P<0,05) without changes in butyrate. The increase of butyrate concentration with

glycerol substitution level is similar that previous studies (Ferraro *et al.,* 2009; Wang *et al.,* 2009) who reported that the butyrate formation in the VFA mixture increased at the expense of acetate when glycerol was supplemented at increasing levels. This fermentation pattern is consistent with other *in vitro* (Bergner *et al.,* 1994; Trabue *et al.,* 2007) and *in vivo* (Defrain *et al.,* 2004; Wang *et al.,* 2009) which confirms the propioneogenic properties of glycerol.

According to Schroder and Südekum (1999), it is likely that glycerol fermentation to ruminal propionate is similar to a fermentable carbohydrate source; the same authors suggested that glycerol of different purities could replace rapidly fermentable starches in diets for ruminants at up to 10% of DM the diet. Our results corroborate the same observation.

Results for butyrate were different from those reported by AbuGhazaleh *et al.* (2011), who observed that butyrate proportions of the total VFA were linearly reduced (P=0,01) with increasing levels of glycerol .

According to Wang *et al.* (2009), glycerol levels increased total VFA production when has added low amounts (i.e., 1,1; 2,2 and 3,3 g/Kg DM) to high forage diets in steers. However, despite the changes in the ratios of VFA in this study, the total VFA production was not affected by glycerol inclusion in the diet (P>0,05).

The data obtained in this study suggest that the use of glycerol may be even more interesting in beef than in dairy cattle, as this could reduce

milk fat as reported by Defrain *et al.* (2004). Meanwhile, Kijora *et al.* (1998) demonstrated that some glycerol can be escaping rumen fermentation, as glycerol content in plasma was significantly higher than in the rumen 12h after the intrarruminal glycerol administration (200 g/d), at days 3 and 7 (glycerol infusion days) compared to day 1 (control, without glycerol). Therefore, Ferraro *et al.* (2009) suggested that glycerol in the treatment and prevention of ketosis in dairy cattle may act either by increasing the pool of propionate or directly as a glucose precursor in the liver.

The rate of liquid passage (Kp), the ruminal volume (RV), the retention time (RT) and the recycling rate (Rec R) were not influenced by the levels of CG in the diet, and the mean values were 10,8% / h; 78,8 l; 9,6h and 2,6 times / day respectively (Table 4). Cereal grains are protected by a protein matrix with structural function, which is heavily concentrated in vitreous endosperm, especially in corn grain (Van Soest, 1994). The complex structure of the corn decreases the starch degradation in the rumen and digestibility when compared to other cereals (McAllister *et al.,* 1990). Thus, as the corn is replaced by CG in the diet by their physical shape and chemistry it is likely to affect crude glycerin ruminal kinetics, increasing the Kp and decreasing the RV and RT. Therefore, it was expected rumen microorganism ferment glycerol faster, but the results were no as expect it. In this sense, Kijora *et al.* (1998) working with steers receiving intra ruminant infusion 200 g glycerol / day, observed that over 85% of the infused glycerol, disappeared in the rumen during the first 2 hours. However, Krehbiel, (2008) suggested that the rate of disappearance of glycerol rumen increased in previously adapted animals.

There were no differences (P>0,05) between treatments for glucose, cholesterol, triglycerides and plasma urea nitrogen values (Table 5). The concentration of glucose in all treatments was higher than the average reported by Pogliani and Junior, (2007) for an adult cattle 74,17 md / dL. It is hypothesized that glycerol may increase plasma glucose levels through its important glucuneogenic activity (Defrain *et al.,* 2004); however data reported in this paper does not demonstrated this .

Because of its gluconeogenic ability, the glycerol has been used for prevention of ketosis, however, the effects of glycerol addition on plasma glucose concentrations are unclear. There are several reports on non-response of plasma glucose from glycerol given supplementation (Chung *et al.,* 2007; Osborne *et al.,* 2009; Rico *et al.,* 2012). Furthermore, Wang *et al.* (2009) offering levels of 0, 100, 200, or 300 g of purified glycerin and Donkin *et al.* (2008), providing 0, 5, 10, or 15% of purified glycerin also observed increase in plasma glucose concentration after glycerin supplementation.

Linke *et al.* (2004) compared delivery methods of glycerol (feeding vs. drenching 800 g) and found drenching to be more efficacious at increasing plasma glucose and insulin concentrations. The amount of glycerol flowed into the abomasum or absorbed across the rumen epithelium when drenched relative to the amount that is fermented when fed appears to determine the gluconeogenicity of glycerol in peripartum dairy cows.

Table 4. Ruminal kinetics depending on crude glycerin levels in beef cattle diets.

Item	Levels of crude glycerin in diet (%)					SE	P
	0	3	6	9	12		
Kp (%/h)	9,7	10,9	10,8	11,0	11,7	0,58	0,24
RV (L)	83,2	80,8	77,8	76,1	76,1	3,79	0,62
RT (h)	10,7	9,4	9,6	9,3	8,9	0,44	0,12
Rec R(times/d)	2,3	2,6	2,6	2,7	2,8	0,14	0,25

Kp (liquid phase) = Passage rate of the liquid phase; RV (Liters) = Ruminal volume; RT = Retention time; Rec R = recycling rate (times/day). SE = standard error of the mean, P = value P.

Table 5. Blood metabolites (mg/dL) depending on crude glycerin levels in beef cattle diets.

Item	Levels of crude glycerin in diet (%)					SE	P
	0	3	6	9	12		
Glucose	75,5	81,2	84,4	86,9	85,4	3,8	0,29
Cholesterol	157,4	163,7	161,4	146,8	141,5	9,0	0,38
Triglycerides	25,2	25,4	26,8	24,5	23,6	1,4	0,62
Plasma urea nitrogen	12,2	11,4	11,6	12,2	11,7	0,6	0,09

SE = standard error of the mean. P = value P.

Defrain *et al.* (2004) showed interaction of plasma glucose concentration and the tendency for greater ruminal butyrate concentrations, it is likely that the ruminal fermentation of glycerol may have increased ruminal butyrate beyond the sample of rumen liquor collected. This is especially true with regard to the inverse relationship between plasma glucose and BHBA (beta-hydroxybutyrate) in cows.

There were no differences between treatments for the plasma urea nitrogen (P>0,05), obtaining an average value of 11,8 mg/dL (Table 5). The results obtained in this study suggest that protein intake in the diet was adjusted to the requirements of the animals in their physiological state. Defrain *et al.* (2004) reported tendencies for a lower milk fat yield and milk urea nitrogen when animals were fed with glycerol. However, in this study with beef cattle this tendency was not observed. The data suggested that the energy availability was similar for both maize and CG. The concentration of plasma glucose, cholesterol and triglycerides did not differ among treatments (P>0,05). The overall average for these variables was 82,7; 154,2 and 25,1 mg/dL respectively. These data are within normal limits for beef cattle, according to Anderson and Rings (2009) and Doornenbal *et al.* (1988).

CONCLUSIONS

The inclusion of 12% CG in the diet of beef cattle based on dry matter decreased the ruminal pH. Also the average and calculated maximum ammonia nitrogen decreases when the level of crude glycerin is increased in the diet. Acetate: propionate ratio decreases with the inclusion of crude glycerin in diets. The proportion of butyrate increases with inclusion of CG in the diet.

It is concluded that CG can be used to feed beef cattle at a level of 12% and can be considered as a good source of alternative energy. It is suggested that the use of crude glycerin in beef cattle diets can improve utilization of ruminal ammonia nitrogen and probably the use of glycerin is potentiated using non-protein nitrogen sources.

LITERATURE REVIEW

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