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Evaluation of crude glycerine inclusion in beef cattle diet: apparent nutrient digestibility and microbial protein synthesis

Evaluación de la inclusión de glicerina cruda en dietas para ganado de carne: digestibilidad aparente de los nutrientes y síntesis de proteína microbiana

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ABSTRACT

Crude glycerine (CG) is an abundant biodiesel by-product and an alternative material for livestock diets. The objective of this study was to evaluate the effects of glycerol supplementation on the digestibility and microbial protein synthesis in Nelore steers. Five ruminally cannulated Nelore breed steers (522±43 kg) were used in a replicated 5×5 Latin Square design to evaluate 0 (control), 3, 6, 9 and 12% of CG in total dry matter of diets during five experimental periods of 21 days. The dry matter intake (DMI), ruminal apparent digestibility (RAD), intestinal apparent digestibility (IAD), total apparent digestibility coefficient (TAD) of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and non-fiber carbohydrates (NFC) were not affected ($P > 0,05$) by CG inclusion. Fecal flow of ether extract (EE) decreased linearly ($P < 0.05$) as the level of CG in the diet increased. However, the CDI and CDT of EE increased linearly ($P < 0.05$) as the level of CG in the diet increased. The inclusion of different levels of CG in the diet showed no significant ($P > 0.05$) effect on microbial protein synthesis. It is concluded that CG may be used in the diet of beef cattle up to 12% and can be considered a good alternative energy.

Key words: biodiesel, co-products, glycerol, ruminants.

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RESUMEN

La glicerina cruda (GC) es un subproducto del procesamiento del biodiesel que se produce abundantemente y puede ser utilizado como un ingrediente alternativo en la nutrición animal. El objetivo de este trabajo de investigación fue evaluar el efecto de la suplementación con glicerina cruda sobre la digestibilidad de los nutrientes y síntesis de proteína microbiana en dietas para novillos Nelore. Cinco novillos raza Nelore canulados (522±43 kg) fueron utilizados en un diseño experimental cuadrado latino 5x5 para evaluar los tratamientos: 0 (control), 3, 6, 9 y 12% of GC en base a la materia seca total de la dieta, durante cinco periodos experimentales de 21 días. La ingestión de material seca (IMS), digestibilidad ruminal aparente (DRA), digestibilidad aparente intestinal(DAI), digestibilidad aparente total (DAT) de la materia seca (MS), materia orgánica (MO), proteína cruda(PC), fibra en detergente neutro(FDN) y carbohidratos no fibrosos (CNF)no fueron afectados ($P > 0,05$) por la inclusión de GC en las dietas. El flujo fecal del extracto etéreo (EE) disminuyó linealmente ($P < 0,05$) a medida que el nivel de GC en la dieta aumentó. Sin embargo, la DAI y DAT del EE aumento linealmente ($P < 0,05$) a medida que aumentó el nivel de GC en la dieta. La inclusión de diferentes niveles de GC en las dietas no afectó ($P > 0,05$) la síntesis de proteína microbiana. Se concluye que la GC puede ser utilizada en las dietas para ganado de carne hasta en un 12% y puede ser considerada como un buen ingrediente energético en las raciones.

Palabras clave: biodiesel, subproductos, glicerol, rumiantes.

INTRODUCTION

Crude glycerine CG is an impure form of glycerine and a major and abundant biodiesel by-product that is being explored for alternative uses such as in livestock diets given its nutritional value. Expansion of biodiesel industry in recent years has lead to a glycerin´s saturated market, thus efforts directed to use its high energy content in animal feed, is an environmentally friendly strategy.

Feedlot cattle or beef feedlot in Brazil has been experiencing major changes in the last years, particularly in the size of enterprises, management and feeding industry. This industry is characterized by the use of diets high in corn. However, corn and another forages are intended for ethanol production and the tendency is a continue increase of corn price and limited use of corn in cattle feeding sector, mainly in the United States. To face those limitations in beef feedlot economy, CG has emerged as a valuable opportunity to reduce feeding costs given its ability to substitute conventional energy foods such as corn grain (Mach *et al.,* 2009). Since the cost of gain for feedlot cattle is considered high, there is great interest in the use of alternative foods that may eventually replace part of grains used in the commercial diets without significant detriment in the physiological, metabolic processes and animal performance (Lage *et al.,* 2010).

Initial studies demonstrated that the inclusion of CG in ruminant´s feed was safe and lead to the complete fermentation of glycerol to propionate (Johns, 1953), and those findings are consistent with recent work (Wilbert *et al.,* 2013). These observations are interesting in the nutrition and beef cattle feeding industry and have been proven by studies on the kinetics using C^{14} – labeled glycerol (Bergner *et al.,* 1995).

According to Trabue *et al.* (2007), CG supply in diets tends to reduce the quantity of available carbon and hydrogen ions for methane production in the rumen, a process that occurs by the reduction of acetate, with the benefits of an improvement in energy use by the animal. Furthermore, glycerol present in CG may increase water retention by the rations, particularly in low humidity environments and increase the palatability of commercial feed because of their sweet fragrance and flavor (Elam *et al.,* 2008). However, evaluation and testing those advantages “in vivo” experiments in needed to construct solid scientific basis for its correct use and potential impact in animal nutrition and beef cattle industry. This study was conducted to evaluate the effects of CG addition in beef cattle diets on the apparent nutrient digestibility and microbial protein synthesis.

MATERIAL AND METHODS

The experiment was performed in the Iguatemi Experimental Farm and the Food and Animal Nutrition Analysis Laboratory, in the Universidad Estadual de Maringá,Brazil. Five 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 gains. A 5 x 5 latin square design was used with five treatments, five periods and five steers per treatment. The experimental periods were of 21 d each with the first15 d intended for adaptation to the experimental diets (Table 1) and the following 6 d for sample collection (foods offered, rejections, feces, rumen liquid, omasaldigesta and urine). 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 rate between fodder and commercial feed was 40:60. The chemical composition of food raw materials used in the experimental diets are shown in Tables 1 and 2.

The GC inclusion in feedlot diets consisted of 0, 3, 6, 9 and 12% based on the total diet´s dry matter. The diets were provided *ad libitum* a complete mixture twice at day (at 08:00 h and at 16:00 h), and the CG was completely mixed with the other ingredients during feed preparation.

In order to determinate total and partial digestibility of dry matter (DM), total dry matter intake (DMI), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and non-fiber carbohydrates (NFC), six samples of omasal chime (~500 ml) were collected daily, throughout the reticulo-omasal orifice by suction, based on the technique described by Leão *et al.* (2005), six samples of feces (~200 g) were collected directly from the rectum. Omasal chyme and feces were collected from day 14, for

Table 1. Chemical composition of food raw materials used in the experimental diets (%)

Nutriment	DM	CP	EE	MM	NDF	ADF
Sorghum silage	25.5	6.9	2.1	5.1	66.0	38.4
Corn	88.7	8.5	5.0	1.3	12.0	3.3
Soybean meal	88.2	53.7	2.7	6.0	9.5	8.9
Wheat flour	87.9	18.9	2.9	3.8	29.6	10.3
Crude glycerine[1]	89.1	0.3	-	6.4	-	-

[1] Content: 73.5% glycerol, 6.0% free fatty acids, 1.52% methanol and 0.05% ethanol (Biopar Industry, Rolândia – Paraná, Brazil). Abbreviations: DM= dry matter; CP= crude protein; EE= extract ether; MM= mineral matter; NDF= neutral detergent fiber; ADF= acid detergent fiber.

6 consecutive days and at 0, 2, 4, 6, 8 and 10 h after the feeding time 8:00 h daily.

To measure the omasal flow and fecal production, 10 g d^{-1}titanium dioxide (TiO_2) was administered directly in the rumen, from day 7 of each period. Collected omasalchyme and feces samples were placed in marked plastic bags and frozen at -20 °C. The samples were thawed, pre-dried for 72 h in an air circulation stove at 55 °C and ground in *Willey* type mills (1 mm mesh). The samples were mixed based on the percentage of dry weight to obtain samples made up of omasal digest and feces per animal/ treatment/period.

Samples of foods used in experimental diets, rejections, omasalchyme and feces were analyzed to determine DM, OM, PC, EE and ashes (AOAC, 1990), NDF and ADF (Van Soest *et al.,* 1991). The values of non-fiber carbohydrates (NFC) and total digestible nutrients TDN were calculated with the Sinffen *et al.* (1992) equations. Samples of omasalchyme and feces were analyzed for titanium concentration (Myers *et al.,* 2004).

Four urine samples per steer were collected during spontaneous urination at 3-4 h after feeding at 08:00 h of each experimental period (days 17 -20). The urine samples were homogenized and filtered through cloth filters, and aliquots of 10 mL were immediately diluted in 40 mL of H_2SO_4 0.036 N (Chen *et al.,* 1995). The pH in the samples was adjusted to values lower than 3 to avoid the bacterial degradation of purine derivatives and the precipitation of uric acid; later, they were stored at -20 °C for analysis of allantoin and uric acid. One subsample of urine without acid was taken on the same day to determine the concentration of creatinine in the urine through the method of final point, using picrate and an acidifying agent following the manufacturer´s recommendations (GoldAnalisa®). Daily volume of urine were calculated based on the creatinine concentration in urine samples and the average levels of creatinine excreted (27.4 mg kg^{-1}) by castrated Nelore reported previously (Barbosa *et al.,* 2006). Those values of urine volume were used to calculate daily excretion of allantoin and uric acid by each animal.

Allantoin in urine was performed by a colorimetry method based on the original technique reported by Fujihara *et al.* (1987), with minor modifications reported later (Chen & Gomes, 1992). The concentration of uric acid was estimated by using a commercial kit (Labtest®). The total excretion of purine derivatives was the result of urinary excretion of allantoin and uric acid.

Absorbed microbial purines (X, mmol d^{-1}) were calculated from the excretion of purine derivatives (Y, mmol d^{-1}), according to the following ecuation: $Y=0.85X + 0.385\ PV^{0.75}$, where 0,85 is the recovery of purines absorbed as purine derivatives and 0,385 $PV^{0.75}$ is the endogenous contribution for purine excretion (Verbic *et al.,* 1990).

The intestinal flow of microbial nitrogenous compounds (Nmic, g N d^{-1}) was calculated from the absorbed microbial purines (Pabs, mmol d^{-1}) according to the following formula:

Nmic=(70*Pabs)/ (0.83*0.116*1000), where 70 corresponds to the N content in purines (mg N^{-1}mmol) and 0,83 is the digestibility of microbial purines; 0,116 is the N purine: N total rate of microorganisms in the rumen (Chen & Gomes, 1992).

The data were analyzed by an ANOVA test using the GLM procedure and when there was a significant effect ($P \leq 0.05$) for the treatment, a polynomial regression analysis was performed using the SAS software (SAS, 2004). The experimental design had the statistical model:

$Yijk=\mu + A_i + P_j + T_k + e_{ijk}$

Where: Y_{ijk}= responses to treatment *k*, animal *i*, in period *j*; μ = treatments mean; A_i=effect of the animal *i*, (1...5); P_j=effect of the period *j*, (1...5); T_k=effect of the treatment *k*, (1...5); e_{ijk}= residual error.

RESULTS AND DISCUSSION

Intake of DM, OM, NDF, CP and NFC, expressed as kg/day were similar among treatments ($P > 0.05$). DMI ranged from 9.53 to 10.11 kg/day and occurred in treatments with 9 and 3% GC inclusion, respectively (Table 2).

Similar results were observed by Mach *et al.* (2009), who evaluated four levels of CG inclusion with 85.7% glycerol, on the performance of Holstein bulls fed with high-concentrate diets and found DMI values of 8.18, 8.19, 8.53 and 8.19 kg/day for 0, 4, 8 and 12% CG levels, respectively. The same authors 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 thatCG 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 result of pectin fermentation.

Inclusion of 16% vs. 0% CG in feedlot steers was reported to decrease DMI in 8.84 and 7.80 kg/day, respectively (Parsons *et al.,* 2009). However, the authors did not report the percentage of glycerol or methanol in CG. In the other hand, Gunn *et al.* (2010) observed that sheep treated with CG (87.5% glycerol), increased DMI in a linear manner and DMI rose from 1.01 to 1.41 kg/day when animals were treated with 0 and 20% CG respectively.

The inclusion of CG in the diet of Nelore steers had no effect ($P > 0.05$) on the CRD, TCD of CID and the DM, OM, CP, NDF and NSC. In this regard, Donkin *et al.* (2009) found that the TCD of the DM and OM increased with the inclusion of CG (0-15%), however, the TCD of the NDF was not affected in our study. Several studies have shown that inclusion of CG in the diet may decrease fiber digestibility. Schröeder & Südekum, (1999) worked with dairy cows receiving diets with forage and 40 to 60% of commercial feed and reported that when 10% of CG are included in diets with high starch, there is a tendency of reducing the digestibility of cell wall, but it does not interfere with TCD of DM and OM.

In vitro experiments had demonstrated that 0.5 to 5.0% pure glycerol inhibit cellulose degradation by cellulolytic bacteria and fungi, respectively (Roger *et al.,* 1992). Although fungi do not play a vital role in ruminal fermentation of high commercial feed diets, the suppression of lytic activity by glycerol may alter the performance of animals or reduce the intake of dry matter and the TCD of the NDF in animals fed fiber rich diets (Schneider, 2010). According to those observations Lage *et al.* (2010) reported that TCD of the NDF had quadratic effect with the inclusion of CG in sheep diets, getting values of 57, 38, 40, 43 and 43% for TCD when CG were 0, 3, 6, 9 and 12%, respectively.

In this study, the inclusion of CG in diets for Nelore steers decreases the flow of fecal EE ($P < 0.05$), results that reflect increased CID and TCD ($P < 0.05$) with higher inclusion of CG (Table 3). Lage *et al.* (2010) fed sheep with similar levels of CG, and noted that the TCD of the EE decreased linearly from 83 to 73% in animals treated with 0 and 12% CG, respectively. The percentage of glycerol in CG was 32.6%, and the authors claimed that the digestibility of EE was influenced by excess of fatty acids present in CG (46.5%). In the present

Table 2. Chemical and feed raw material composition of experimental diets based on DM.

Nutriment	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 (%)				
CP	13.5	13.5	13.5	13.5	13.5
EE	3.1	3.2	3.2	3.2	3.2
NDF	36.7	36.4	36.1	35.8	35.5
NFC[2]	43.1	43.2	43.6	43.9	44.2
TDN[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. PC= crude protein; EE= ether extract; NDF= neutral detergent fiber, NFC and TDN= non-structural carbohydrates and total nutrients digestible calculated by Sniffen *et al.,* (1992); [3]NEm= net energy for maintenance, and [4]NEg= net energy for gain, calculated by NRC (2000).

study, the percentage of fatty acids in CG was only 6%, it could be assumed that the increase in CID and TCD can be due to decreased lipolysis in the rumen. Krueger *et al.* (2010) observed 48 and 77% reduction in lipolysis when 2 and 20% of glycerol, respectively is included in *in vitro* experiments. This fact may causes a greater flow of EE to the gut, thus improving the digestibility.

Our results also indicated that the inclusion of CG in the diet had no effect ($P > 0.05$) on the urinary volume allantoin, ureic acid, total purine, purines absorbed, microbial nitrogen and efficiency of microbial protein synthesis (Table 4).

According to Gurtler *et al.* (1987), the urinary volume of one adult bovine ranges from 5 to 10 L/day, in agreement with the values observed for urine production in all treatments. The average urine volume observed in this experiment (6.72 L/day) was superior to the volume reported by Barbosa *et al.* (2006), for castrated Nelore males (4,67 L/day) and lower those observed by Chizzotti *et al.* (2006), in heifers with 453 kg (17.47 L/day) mean body-weight.

The mean urinary excretion of allantoin and ureic acid obtained in this study (149 mmol/day) were higher than those obtained by Wang *et al.* (2009), who evaluated different levels of (100, 200 and 300 g/day) glycerol in the diet of

Table 3. Digestibility parameters of diets with different CG inclusion levels. Abbrevaitions: Intake (INT), omasal flow (OF), fecal flow (FF), coefficient of apparent ruminal digestibility (CRD), coefficient of apparent intestinal digestibility (CID) and total apparent digestibility coefficient (TCD), DM, OM, CP, NDF, EE and NSC, depending on the levels of CG in the diet of beef cattle.

Variable		Levels of CG in the diet (%)					SE	P<
		0	3	6	9	12		
DM	INT g/day	9643	10112	9926	9532	9858	208.8	0.35
	OF - g/day	5719	5760	5473	5489	5056	316.3	0.56
	FF - g/day	4014	4118	3806	3837	3853	152.5	0.56
	CRD - %	40.7	43.0	44.9	42.4	48.7	2.8	0.48
	CID - %	29.8	28.5	30.5	30.1	23.7	4.6	0.63
	TCD - %	58.4	59.2	61.5	59.8	61.0	1.5	0.58
OM	INT g/day	9139	9564	9370	8982	9273	196.6	0.34
	OF - g/day	5012	5031	4758	4753	4374	282.5	0.52
	FF - g/day	3531	3598	3326	3361	3339	138.8	0.54
	CRD - %	45.2	47.4	49.2	47.1	52.8	2.6	0.47
	CID - %	29.5	28.5	30.1	29.3	23.7	4.6	0.73
	TCD - %	61.4	62.3	64.3	62.7	64.1	1.4	0.58
CP	INT g/day	1303	1397	1358	1307	1340	27.8	0.16
	FO - g/day	1002	1030	1052	1026	984	61.3	0.96
	FF - g/day	483	520	480	477	513	16.2	0.24
	CRD - %	23.3	26.5	22.5	21.5	26.6	4.0	0.52
	CID - %	51.8	49.5	54.4	53.5	47.9	3.0	0.69
	TCD - %	62.9	62.9	64.8	63.5	61.7	1.2	0.51
NDF	INT g/dia	3426	3521	3437	3266	3419	93.6	0.46
	OF - g/dia	2944	3011	2784	2817	2647	174.6	0.62
	FF - g/dia	2403	2449	2310	2374	2378	95.2	0.88
	CRD - %	14.1	14.4	19.0	14.0	22.6	4.6	0.15
	CID - %	18.4	18.8	17.0	15.7	10.3	6.3	0.79
	TCD - %	29.9	30.3	32.8	27.4	30.7	2.9	0.75
EE	INT g/day	298	316	313	300	314	9.0	0.49
	OF - g/day	279	282	295	274	257	20.3	0.77
	FF - g/day	75	65	55	49	46	4.6	0.01
	CRD - %	6.3	10.7	5.8	8.7	18.1	5.6	0.55
	CID - %	73.1	76.9	81.3	82.0	82.2	1.6	0.01
	TCD - %	74.8	79.4	82.4	83.6	85.4	1.3	0.01
NSC	INT g/dia	4273	4518	4463	4322	4435	78.7	0.22
	OF - g/dia	787	706	627	635	486	107.3	0.21
	FF - g/dia	570	564	482	461	401	52.3	0.18
	CRD - %	81.6	84.3	85.9	85.3	89.0	2.4	0.33
	CID - %	27.5	20.0	23.0	27.2	17.4	4.3	0.68
	TCD - %	86.8	87.4	89.1	89.4	90.8	1.2	0.18
	TDN[1]	62.8	63.9	66.1	64.6	66.1	1.4	0.41

[1] Calculated according Sniffen *et al.* (1992). SE = standard error of average. P = P value.

Table 4. Efficiency of microbial protein synthesis in cattle fed increasing levels of CG.

Variable	Levels of CG in the diet (%)					SE	P<
	0	3	6	9	12		
UV	6.07	5.96	7.10	8.30	6.16	0.56	0.15
ALL	144.06	151.95	155.36	156.31	138.58	6.89	0.39
UAc	9.36	9.50	11.27	11.87	9.79	0.96	0.29
PD	153.42	161.45	166.63	168.18	149.38	7.40	0.35
Pabs	131.68	140.61	146.90	147.35	126.38	8.67	0.38
Nmic	95.73	102.22	106.79	107.12	91.87	6.30	0.38
Efic.	125.53	136.31	140.62	136.82	116.59	8.47	0.30

UV – urine volume (L/day); ALL – Allantoin (mmol/day); UAc – Uric acid (mmol/day); PD – purine derivatives (mmol/day); Pabs – Purines absorbed (mmol/day); Nmic – microbial nitrogen (g/day); Efic. – Efficiency of microbial protein synthesis (g CP microbial/kg de TDN consumed).

Simental steers and observed 58, 64 and 60 mmol/day of allantoin excretion, respectively, and 7.38, 7.44 and 7.32 mmol/day of ureic acid in diets of steers, respectively.

The percentage of uric acid in the purine derivatives (PD) ranges from 15 to 20% and it was very constant in the same animal, but variable between animals (Chen & Gomes, 1992). However, in this experiment the percentage of uric acid ranged from 3.9 to 5.3%, and it was lower than those observed by Castañeda *et al.* (2009), whom reported 6.4 to 10.4% in Holstein steers and the mean value of 8.25% in total purine in uric acid in heifers with 523 Kg mean body-weight reported by Chizzotti *et al.* (2006).

The average value for efficiency in microbial protein synthesis from protein microbial was 131.17 g/kg of TDN intake and this value was similar to that reported by Magalhães *et al.* (2005) whom reported 133.55 CP mic g/kg of TDN intake in steers fed diets with increasing levels of urea. However, Nascimento *et al.* (2010) obtained values between 85 and 103 g PBmic/kg TDN intake in crossbred steers fed different energy sources. Those values are lower those observed in this study and indicate that. Finally, the values observed in Nelore steers in this study were close to those (130.0 g CP mic/kg TDN) proposed by NRC (2001) and higher to those reported by Valadares Filho *et al.* (2006) for cattle (120.0 g CP mic/kg of TDN) in tropical conditions. Thus, it can be inferred that the estimated values for microbial synthesis are between normal ranges and support the theory that CG inclusion does not adversely alter the population of microorganisms in the rumen micro-environment.

CONCLUSIONS

The inclusion of 12% crude glycerin in the diet of beef cattle, do not influence the ingestion of dry matter, partial and total digestibility of nutrients and microbial protein synthesis. The intestinal and total digestibility of lipids improved with the inclusion of dietary CG. It is concluded that crude glycerin with low ethanol levels could be a good alternative energy feedstuff, and may be added up to 12% of total DM to achieve a partial replacement of ground corn grain in the diet of beef cattle.

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