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ANIMAL PRODUCTION

Inclusion of crude glycerin with different roughages changes ruminal parameters and *in vitro* gas production from beef cattle

Inclusão de glicerina bruta a diferentes volumosos altera os parâmetros ruminais e a produção de gases *in vitro* de bovinos confinados

Josimari Regina Paschoaloto[I*] Jane Maria Bertocco Ezequiel[I] Marco Túlio Costa Almeida[I] Vanessa Ruiz Fávaro[II] Antonio Carlos Homem Junior[III] Vanessa Barbosa de Carvalho[IV] Henrique Leal Perez[I]

ABSTRACT

The increasing availability of crude glycerin from biodiesel production has generated great stock in the industries. To solve this problem, crude glycerin is being used as an energy source to replace corn in livestock diets. This study evaluated the effects of the association of crude glycerin (10% on DM of diets) with different roughages in Nellore cattle diets on ruminal pH and ammonia, degradability, digestibility of dry matter and nutrients, and greenhouse gas production. Six ruminally cannulated Nellore steers were assigned to a 6×6 Latin square design. The following treatments were evaluated: Hydrolyzed Sugarcane associated or not with crude glycerin, Corn Silage associated or not with crude glycerin or Tifton-85 Hay associated or not with crude glycerin. Association of crude glycerin with roughages did not affect the rumen ammonia concentration and pH and dry matter intake, but reduced the intake of NDF for diets with Hydrolyzed Sugarcane and Corn Silage and reduced the digestibility of DM, OM, NDF, EE, CNF and starch and decreased the effective degradation at the rate of 8% h^{-1} for diets with Tifton-85 Hay. The association crude glycerin with Hydrolyzed Sugarcane reduced the production of CH_4 and CO_2 in mL g^{-1} of DM. The inclusion of crude glycerin affects differently nutrient utilization in diets with Corn Silage, Hydrolyzed Sugarcane or Tifton-85 hay. Moreover, promotes mitigation of greenhouse gases in diets with Hydrolyzed Sugarcane. Association of crude glycerin with Corn Silage in Nellore cattle diets showed better conditions of ruminal fermentation and utilization of nutrients.

Key words: *biodiesel, byproducts, degradability, digestibility.*

RESUMO

A crescente disponibilidade de glicerina bruta proveniente da produção de biodiesel tem gerado grande estoque nas indústrias. Para resolver esse problema, a glicerina bruta está sendo utilizada na alimentação animal como fonte energética. O objetivo deste estudo foi avaliar os efeitos da associação da glicerina bruta (10% da MS das dietas) com diferentes volumosos sobre o pH e amônia ruminal, degradabilidade, digestibilidade da matéria seca e nutrientes, e produção de gás de efeito estufa. Seis novilhos da raça Nelore foram distribuídos aleatoriamente em um quadrado latino 6×6. Os tratamentos avaliados foram cana-hidrolisada associada ou não à glicerina, silagem de milho associada ou não à glicerina ou feno de Tifton 85 associado ou não à glicerina. A concentração de amônia ruminal e pH não foram afetados pela associação glicerina bruta com volumosos e consumo de matéria seca, mas reduziu o consumo de FDN para dietas com cana-hidrolisada e silagem de milho, e reduziu a digestibilidade de MS, MO, FDN, EE, CNF e amido para dietas com Feno de Tifton 85, além de reduzir a degradação efetiva na taxa de 8% h^{-1} nesta dieta. A associação glicerina bruta com cana-de-açúcar hidrolisada reduziu a produção de CH_4 e de CO_2 em mL g^{-1} de MS. A associação da glicerina bruta afeta a utilização de nutrientes, independente do volumoso utilizado. Além disso, promove a mitigação de gases de efeito estufa em dietas com cana-de-açúcar hidrolisada e, quando associada à silagem de milho, mostrou melhores condições de fermentação ruminal e utilização dos nutrientes.

Palavras-chave: *biodiesel, coprodutos, degradabilidade, digestibilidade.*

INTRODUCTION

In recent years, biodiesel production has been growing exponentially worldwide, leading to increased stocks of crude glycerin, a once valuable

[I]Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, SP, Brasil. E-mail: jpaschoaloto@yahoo.com.br. *Corresponding author.
[II]Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, Estação Experimental de Lages, Lages, SC, Brasil.
[III]Centro Estadual de Educação Tecnológica Paula Souza, Faculdade de Tecnologia, Taquaritinga, SP, Brasil.
[IV]Faculdade de Agronomia, Universidade José do Rosário Vellano (UNIFENAS), Campus Universitário, Alfenas, MG, Brasil.

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by-product that is now considered a residual with disposal costs (YAZDANI & GONZALEZ, 2007). As the production of biodiesel grows, the amount of crude glycerin generated increases, and its utilization will become interesting. In this regard, the inclusion of crude glycerin to animal diets has been explored as a route to use this by-product in an economically and environmentally correct way.

Research on the inclusion of crude glycerin in animal diets in majority evaluated its effect on performance, weight gain, meat quality and ruminal fermentation (WANG et al., 2009; PARSONS et al., 2010; ABO-EL-NOR et al., 2010; AVILA-STAGNO et al., 2011; RAMOS & KERLEY, 2012; AVILA-STAGNO et al., 2013), but little research has been done to evaluate the effect of glycerin when associated with different kind of roughages on absorption of nutrients and gases production, since each diet utilizes a roughage source. Thus, the effect of crude glycerin on the use of diets with different types of fiber remains unknown.

This study hypothesized that roughage source changes the use of nutrients of diets containing crude glycerin. This study evaluated which fiber source allows a better use of nutrients and reduction on *in vitro* gas production (CO_2 and CH_4) in the presence of crude glycerin. The results of this research have potential to maximize the use of crude glycerin in animal feeds in order to facilitate the use of this biodiesel by-product.

MATERIALS AND METHODS

Six ruminally cannulated Nellore steers averaging 24 months of age and 400kg BW were assigned to a 6×6 Latin square design. The diets contained equal protein (12.4% crude protein on dry matter basis) and energy (2.6Mcal metabolizable energy kg^{-1} on dry matter basis) concentrations and were formulated to meet the requirements of Nellore steers in feedlot according to NRC (1996). The diets contained 50% roughages and 50% concentrate (sunflower meal, corn grain, soybean hulls, mineral and vitamin supplement) with or without crude glycerin (Table 1). The following treatments were evaluated: Hydrolyzed Sugarcane associated or not with crude glycerin (HSG and HS, respectively), Corn Silage associated or not with crude glycerin (CSG and CS, respectively) or Tifton-85 Hay (Bermuda grass) associated or not with crude glycerin (THG and TH, respectively). The inclusion of crude glycerin was 10% on dry matter of diets and the crude glycerin contained 86% of glycerol. Animals were fed twice daily (0800 and 1600h) for *ad libitum* intake for 21d in each experimental period (2wk adaptation and 1wk data collection).

For determination of daily dry matter intake (DMI), refusals were collected and weighed daily before feeding. Apparent digestibility of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), non-fibrous carbohydrates (NFC) and starch (STCH) were determined using the indigestible neutral detergent fiber (iNDF) as internal marker, as described by CASALI et al. (2008).

In situ degradation kinetics in the rumen was measured using the nylon bag technique (ØRSKOV & MCDONALD, 1979). Bags were suspended in the rumen of each steer for 0, 6, 12, 24, 48, 72, 96, 120 and 144h. The disappearance of DM were determined using the non-linear model described by ØRSKOV & MCDONALD (1979) to determine the constants and potential degradation (PD) according to the exponential model: $PD=A+B(1-e^{\wedge}k_dt)$, where A is the soluble fraction (g kg^{-1}; fraction washed out at t=0), B is the insoluble degradable fraction (g kg^{-1}), k_d is the fractional degradation rate (h^{-1}) and t is the time (h). The effective degradability (ED; g kg^{-1}) was calculated from the aforementioned parameters assuming fractional passage rates (k_p) of 2, 5 and 8% h^{-1}: $ED=A+B(k/(k+k_p))$.

The methodology adopted for determination of *in vitro* gas production was adapted from PEREIRA et al. (2006), and we evaluated the production within 24 hours of CH_4 and CO_2 in mL/g DM. Gas concentration was analyzed in a gas chromatograph (Trace GC UltraTM, Thermo Scientific).

Rumen fluid samples were collected on d 15 of each experimental period, at -1, 0, 2, 4, 6, and 8h after feeding. Approximately 500g of ruminal content of each animal adapted to each experimental diet were collected from the dorsal and ventral rumen, and strained through four layers of cheese cloth to separate liquid and solid phases. The pH was determined immediately after rumen fluid sampling by using a digital pH meter and ammonia concentrations were determined using a micro- Kjeldahl device (VIEIRA, 1980), using 5mL 2N KOH, and a distillation flux of 2mL min^{-1}. The distilled sample was dropped in 10mL boric acid solution (2mol L^{-1}) and then titrated with 0.005N HCl.

Analysis of DM, OM, MM, CP and EE were performed according to AOAC (1995), NDF, ADF according to VAN SOEST & WINE (1967), STCH according to HENDRIX (1993) and NFC calculated by difference [100-(% CP+% NDF+% ash+% EE)].

Table 1 - Percentage of feed ingredients and chemical composition of experimental diets.

Item	Treatments[1]					
	HS	HSG	CS	CSG	TH	THG
Ingredients, % DM						
Hydrolyzed sugarcane	50.0	50.0	-	-	-	-
Corn silage	-	-	50.0	50.0	-	-
Tifton-85 hay	-	-	-	-	50.0	50.0
Corn grain	13.4	7.4	24.5	13.5	33.3	18.3
Soybean hulls	11.5	3.0	10.1	6.8	8.0	10.0
Sunflower meal	24.5	28.7	13.6	17.6	7.7	10.7
Limestone	-	-	1.0	1.2	-	-
Urea	0.1	0.35	0.3	0.35	0.5	0.5
Crude glycerin	-	10.0	-	10.0	-	10.0
Mineral and vitamin supplement	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition, % DM						
CP	12.0	12.7	12.5	12.6	12.2	12.1
EE	1.3	1.0	3.2	2.8	2.3	1.8
NDF	47.5	42.9	41.0	39.2	52.1	52.8
ADF	31.7	28.8	26.1	25.6	29.0	30.7
NFC	34.3	33.4	39.8	36.4	29.5	22.6
STCH	15.7	15.5	29.2	28.3	20.7	19.2
ME (Mcal/ kg)[2]	2.4	2.4	2.7	2.7	2.6	2.5

[1]HS = Hydrolyzed Sugarcane; HSG = Hydrolyzed Sugarcane with crude glycerin; CS = Corn Silage; CSG = Corn Silage with crude glycerin; TH = Tifton-85 Hay; THG = Tifton-85 Hay with crude glycerin; [2]Metabolizable energy (Mcal kg^{-1} DM) estimated by the NRC (1996).

The variables were analyzed as a 6×6 Latin square design in a 3×2 factorial arrangement (three roughages and with or without crude glycerin). Data were analyzed using the PROC MIXED (SAS, version 9.2) to account for effects of square, period within square, animal within square and treatment. Treatment was considered a fixed effect; square, period within square, and animal within square were considered random effects. Data for pH, rumen ammonia and degradability were summarized by sampling time and then analyzed using the same mixed model but with time included as a repeated measure using compound symmetry. Contrasts were conducted to test the effects of association of crude glycerin with roughages (HSG *vs* HS, CSG *vs* CS and THG *vs* TH) and the significance was set at P<0.05.

RESULTS

Rumen ammonia concentration and pH were not affected by the association of crude glycerin with roughages (P=0.092 and 0.815, respectively), the mean values for all treatments were 20.57mg dL^{-1} and 6.64, respectively (Table 2). The association between crude glycerin and Hydrolyzed Sugarcane reduced the production of CH_4 (P=0.017) and CO_2 (P=0.004) in mL g^{-1} of DM. The association of crude glycerin with roughage did not affect dry matter intake (P>0.05), however, reduced the intake of EE and NFC for all treatments, and reduced the intake of CP and NDF for diets with Hydrolyzed Sugarcane and reduced the intake of NDF for diets with Corn Silage (P<0.05).

The association of crude glycerin with Tifton-85 Hay reduced the digestibility of DM, OM, NDF, EE, CNF and STCH (P<0.05), and the association with Hydrolyzed Sugarcane reduced the digestibility of NDF and STCH (P<0.05, Table 3). The results showed that the association of crude glycerin with different roughages did not affect the ruminal degradation kinetics. We only observed a reduction in the effective degradation at 8% h^{-1} rate, when crude glycerin was associated with Tifton-85 Hay (P=0.038).

DISCUSSION

The association of crude glycerin did not affect the ruminal ammonia concentrations and pH, independent of the roughage used. Possibly, it was due to the roughage: concentrate ratio of the diets. The

Table 2 - Effects of crude glycerin included or not with different roughages on feed intake (kg day^{-1}), ruminal parameters and gas production in Nellore steers.

Item	Treatments[1]						SEM[2]	Contrast[3], *P*-value		
	HS	HSG	CS	CSG	TH	THG		1	2	3
DM	7.0	6.5	9.1	8.7	6.7	6.5	7.0	0.383	0.468	0.730
OM	6.3	5.8	8.4	8.1	6.1	5.9	6.3	0.312	0.502	0.724
CP	0.8	0.7	1.9	1.6	1.0	0.9	0.8	0.036	0.001	0.091
NDF	3.6	3.2	4.9	4.0	4.1	4.0	3.6	0.140	0.007	0.520
ADF	2.2	2.0	3.0	2.6	4.0	4.0	2.2	0.360	0.062	0.951
EE	0.1	0.1	0.3	0.3	0.2	0.2	0.1	0.0002	<0.0001	0.0002
NFC	2.9	2.3	3.1	2.6	2.2	1.3	2.9	0.003	0.008	0.0003
STCH[4]	1.0	0.9	3.6	3.6	1.1	1.1	1.0	0.426	0.326	0.727
pH	6.8	6.9	6.6	6.5	6.4	6.6	6.8	0.092	0.065	0.068
NH_3-N[5]	17.5	12.5	28.6	27.4	17.8	17.3	0.82	0.052	0.154	0.256
CH_4[6]	23.9	17.4	15.1	16.2	16.7	16.2	1.01	0.017	0.784	0.785
CO_2[6]	95.9	70.6	62.8	64.8	57.2	54.2	4.35	0.004	0.830	0.728

[1]HS = Hydrolyzed Sugarcane; HSG = Hydrolyzed Sugarcane with crude glycerin; CS = Corn Silage; CSG = Corn Silage with crude glycerin; TH = Tifton-85 Hay; THG = Tifton-85 Hay with crude glycerin; [2]SEM = Standard error of the mean. [3]Contrast = 1 = HS × HSG; 2 = CS × CSG; 3 = TH × THG. [4]STCH = Starch. [5]NH_3-N = Ruminal ammonia concentration, mg dL^{-1}. [6]CH_4 = Methane (mL g^{-1} of DM); CO_2 = Carbon dioxide (mL g^{-1} of DM).

use of 50% of concentrate in the diets hardly affects the ruminal fermentation. All values obtained in this study remained within the range considered as optimal for the fermentative activity of bacteria, between 6.0 and 6.4 (VAN SOEST, 1994). Regarding the ruminal ammonia concentration, all the results obtained were above 5mg dL^{-1}, value considered as a minimum for proper rumen fermentation (SATTER & SLYTER, 1974). In agreement with our results, RAMOS & KERLEY (2012) did not find different concentration of ammonia nitrogen in cattle fed with crude glycerin.

The reduction observed in the intake EE and CNF for all treatments with inclusion of crude glycerin may be because crude glycerin does not contain cell wall and there are low levels of EE. The crude glycerin used in this study was consisted of 86% glycerol, 89% DM, 0.2% EE, 1.2% CP, 0% NDF, 6% NaCl, and less than 0.01% methanol. The same explanation may be used for the reduction on the intake of CP and NDF observed in the treatment with Corn Silage, and reduction on the intake of NDF in the treatments with Hydrolyzed Sugarcane when crude glycerin was included.

Reduction observed in the digestibility of DM, OM, NDF, EE, NFC and STCH in treatments with Tifton-85 Hay and reduction in the digestibility of NDF and STCH in treatments with Hydrolyzed Sugarcane when crude glycerin were included may be explained by the deleterious selection of fibrolytic microorganisms, which are known to be sensitive to glycerin. Previous studies have shown that crude glycerin provides selection of rumen microorganisms, mainly fibrolytic ones (ABO-EL-NOR et al., 2010), which can affect the NDF digestibility and authors attributed the reduction in NDF digestibility to the decrease in DNA concentration from bacteria ***Selenomonas ruminantium*** and ***Butirivibrio fibrosolvens***, caused by the addition of crude glycerin to the diet. ROGER et al. (1992) also reported that glycerin decreases microbial growth, cell membrane permeability, and adhesion of bacteria in feed. These facts may have impaired not only the digestibility of NDF but also other nutrients. Therefore, it is plausible that glycerin generates deleterious effects in digestibility of high fiber diets. Tifton-85 proved to be of poor quality, with greater NDF, lower NFC and ED in 8% h-1 (Table 1 and 3). However, we did not observe a decline in digestibility of DM and others nutrients in treatments with Corn Silage when crude glycerin was added. This diet provided better ruminal parameters for microbial growth and utilization of nutrients in diets.

The *in vitro* reduction of gases production (CH_4 and CO_2, Table 2) when crude glycerin was added to the diets with Hydrolyzed Sugarcane can be associated with the change in the profile of gases produced with the inclusion of such by-product (REMOND et al., 1993). Low intake of NDF observed

Table 3 - Effects of crude glycerin associated or not with different roughages on apparent digestibility (%) and ruminal degradation kinetics in Nellore steers.

Item	Treatment [1]						SEM[2]	Contrast[3], *P*-value		
	HS	HSG	CS	CSG	TH	THG		1	2	3
Apparent digestibility (%)										
DM	80.5	78.7	79.8	78.9	72.0	67.0	0.89	0.051	0.522	0.001
OM	81.8	80.0	80.6	80.2	73.0	67.9	0.92	0.156	0.740	0.0005
CP	72.6	73.7	73.1	72.3	75.2	76.5	0.91	0.668	0.748	0.593
NDF	79.6	77.1	76.9	75.2	62.4	53.4	0.65	0.046	0.387	0.001
ADF	76.1	73.7	63.0	61.2	55.0	53.2	1.58	0.352	0.487	0.475
EE	83.1	81.8	83.9	85.7	80.7	75.4	1.61	0.341	0.194	0.001
NFC	94.2	94.2	90.6	90.4	90.7	84.1	0.62	0.959	0.790	0.001
STCH [4]	87.3	85.6	93.2	91.8	91.0	79.7	0.83	0.044	0.080	0.001
Ruminal degradation kinetics										
PD %[5]	73.4	72.1	78.9	80.1	68.0	67.7	1.07	0.542	0.595	0.871
ED 2%[6]	60.7	61.1	63.3	63.5	47.7	46.1	1.34	0.767	0.854	0.210
ED 5%[7]	54.3	55.3	54.7	57.9	36.0	34.3	1.62	0.261	0.852	0.059
ED 8%[8]	51.6	52.8	51.0	51.2	30.7	29.2	1.76	0.094	0.712	0.038
k_d[9]	2.33	2.48	2.71	2.42	3.04	2.55	0.11	0.563	0.263	0.062

[1]HS = Hydrolyzed Sugarcane; HSG = Hydrolyzed Sugarcane with crude glycerin; CS = Corn Silage; CSG = Corn Silage with crude glycerin; TH = Tifton-85 Hay; THG = Tifton-85 Hay with crude glycerin; [2]SEM = Standard error of the mean. [3]Contrast = 1 = HS × HSG; 2 = CS × CSG; 3 = TH × THG. [4]STCH = Starch. [5]PD = Potential degradation; [6]ED 2% = Effective degradability 2% h^{-1}; [7]ED 5% = Effective degradability 5% h^{-1}; [8]ED 2% = Effective degradability 8% h^{-1}; [9]k_d = passage rate.

in this diet can explain these results (Table 2). With the reducing intake of NDF, there may be a reduced number of microorganisms involved in fiber digestion, leading to reduction of acetic acid, which is a by-product of fiber digestibility, and consequently reduced methane production. This result demonstrated that the effect of glycerol is dependent on its interaction with roughage and concentrate. Furthermore, may affect animal performance by lower digestibility of nutrients.

CONCLUSION

The inclusion of crude glycerin (10% DM) with Hydrolyzed Sugarcane promotes a reduced production of CH_4 and CO_2, and when included with Tifton-85 Hay reduced the digestibility of nutrients. The addition of crude glycerin to Corn Silage in Nellore cattle diets showed better conditions of ruminal fermentation and utilization of nutrients.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The cannulation procedures and the use of the ruminally cannulated animals in these experiments were approved by the Animal Welfare and Ethics Commission from Universidade Estadual Paulista (Protocol 028066/12).

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