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SUBSTITUTING SORGHUM GRAIN WITH CRUDE GLYCEROL IN DIETS FOR BEEF CATTLE

(*Substituição do sorgo grão pelo glicerol não refinado em dietas para bovinos de corte*)

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ABSTRACT: The expansion of the biodiesel industry has created opportunities for crude glycerol use in beef cattle diets. The objective of this study was to determine the effects of substituting sorghum grain with crude glycerol on dry matter intake (DMI), weight gain and feed intake pattern of 28 non-castrated Nelore males with initial body weight (BW) of 441 ± 40.2 kg and 21.5 ± 0.5 months of age housed in individual or collective pens. The experiment lasted 98 days (June 29 through October 5, 2015), with a 14 day-period of adaptation and 84 days for data collection. Crude glycerol was included at 15% of the ration dry matter as a replacement for sorghum grain. Orts were collected and weighed daily, and DMI was calculated by difference between feed offered and feed refused. Feed intake pattern was determined every two weeks after the beginning of the study in three intervals relative to feed delivery (0-4, 4-10, and 10-24 hours post-feeding). Animals' BW was obtained on days 1, 13, 27, 41, 55, 69, and 83 days of the trial after a 12-hour solid fasting. Crude glycerol did not alter ($P>0.05$) DMI, weight gain, hot carcass weight and dressing percentage compared with the control diet. A treatment \times days of experiment response ($P<0.05$) occurred due to a decreased DMI in animals fed crude glycerol during the first 14 days of the trial. Crude glycerol-fed animals decreased ($P<0.05$) the neutral detergent fiber (NDF) intake pattern in individual and collective pens. Crude glycerol can be recommended at 15% of the total DM ration as a sorghum grain replacement in diets fed to Nelore animals finished in feedlot.

Key words: biodiesel; byproduct; energy; feedlot; Nelore.

RESUMO: A expansão na indústria de biodiesel tem criado oportunidades para o uso do glicerol não refinado em rações de bovinos de corte. Objetivou-se neste estudo determinar os efeitos da substituição do sorgo grão pelo glicerol não refinado sobre o consumo de matéria seca (CMS), ganho de peso e padrão de alimentação de 28 machos Nelore não castrados com peso corporal (PC) inicial de $441 \pm 40,2$ kg e $21,5 \pm 0,5$ meses de idade alojados em baias individuais ou coletivas. O experimento teve duração de 98 dias (29 de junho a 5 de outubro de 2015) com período de adaptação de 14 dias e 84 dias para coleta dos dados. Incluiu-se o glicerol não refinado em 15% da MS da ração como substituto do sorgo grão. As sobras foram coletadas e pesadas diariamente e o CMS foi calculado pela diferença entre o oferecido e sobras. Determinou-se o padrão de alimentação a cada 2 semanas após o início do estudo em 3 intervalos relativos ao início da alimentação

(0-4, 4-10 e 10-24 horas pós-alimentação). Obteve-se o PC dos animais nos dias 1, 13, 27, 41, 55, 69 e 83 dias da pesquisa após jejum de sólidos de 12 horas. O glicerol não refinado não alterou ($P>0,05$) o CMS, ganho de peso, peso da carcaça quente e rendimento de carcaça comparado à dieta controle. Houve resposta ($P<0,05$) de tratamento \times dias do experimento devido à redução do CMS nos animais alimentados com glicerol não refinado nos primeiros 14 dias da pesquisa. Os animais alimentados com glicerol não refinado reduziram ($P<0,05$) o padrão de consumo de fibra em detergente neutro (FDN) nas baias individuais e coletivas. O glicerol não refinado pode ser recomendado em 15% do total da MS da ração como substituto do sorgo grão em dietas para animais Nelore terminados em confinamento.

Palavras-chave: biodiesel; confinamento; energia; Nelore; subproduto.

RETRACTED

INTRODUCTION

The world's population growth rate has been declining over the past years, but the estimates still point out a total of 8.5 billion people by 2030 (Department of Economic and Social Affairs of the United Nations, 2017). Although in a lower growth rate, such an amount of human beings will continuously increase the demand for more food. Therefore, in order to increase the productivity of the beef industry, it is necessary more research to understand the potential of byproducts that can replace traditional energy sources used in beef cattle rations, which may contribute to mitigate the utilization of natural resources for grain production.

The expansion of the biodiesel industry across the world has increased the stocks of crude glycerol, which has exceeded the capacity of the pharmaceutical, cosmetic, and food industries to use refined glycerol (Ayoub and Abdullah, 2012). Only the Brazilian National Production of biodiesel in 2017 was 3.8 million m³ (ANP, 2017). Reasons for such a big volume are based on the great diversity of oilseed crops for biodiesel production and the current mandatory addition of 8% (v/v) of biodiesel to diesel oil (ANP, 2017). Consequently, there is a great potential for the utilization of crude glycerol as an alternative energy source in diets for beef cattle, although certain on-farm bottlenecks, like storage, sequence of mixing and adequate homogenization with diet ingredients, distance between the biodiesel industry and the feedlot, and price competitiveness compared with classical energy feeds may restrain the use of crude glycerol.

Crude glycerol is a byproduct of the biodiesel industry that has been currently produced by a reaction that utilizes a base-catalyzed transesterification of vegetable oils or animal fat in the formation of methyl and ethyl fatty acid esters in the production of biodiesel, while crude glycerol is left

behind (Ayoub and Abdullah, 2012). For each 100 g of soybean oil input there is a yield of 12.25 g of crude glycerol, which is considered to be high in terms of a byproduct (Thompson and He, 2006).

The U.S. legislation has assigned a GRAS (generally recognized as safe) status to glycerol as a feed ingredient in animal rations (FDA, 2006), although the category issued by the FDA (2006) was for refined glycerol. On the other hand, crude glycerol may have contaminants, including water, salts and methanol (Thompson and He, 2006). According to the FDA (2006), crude glycerol should contain no more than 150 mg of methanol/kg of glycerol, therefore levels above this limit are inappropriate for animal feeding.

Recent studies have demonstrated the potential of feeding crude glycerol to beef cattle (Bartoň *et al.*, 2013; Egea *et al.*, 2014; Eira *et al.*, 2014; van Cleef *et al.*, 2014; Pairey *et al.*, 2015; Fávaro *et al.*, 2015), but none of them have reported data about the substitution of sorghum grain with crude glycerol. We hypothesized that crude glycerol could partially replace sorghum grain in diets for beef cattle in a feedlot without compromising animal performance.

The objective of the present experiment was to determine the effects of substituting sorghum grain with crude glycerol in rations fed to beef cattle on feed intake, weight gain, and feed intake pattern.

MATERIALS AND METHODS

Experimental site

The present study was conducted at the Dairy and Beef Research and Education Center of the "Instituto Federal de Educação, Ciência e Tecnologia Goiano" (IF Goiano), Iporá, Goiás State, Brazil from June 29 through October 5, 2015. The experiment lasted 98 days, with 14 days of adaptation of the animals for the new facilities and

experimental diets, and 84 days for data collection.

Animals and dietary treatments

Twenty-eight non-castrated Nelore males with initial body weight (BW) of 441 ± 40.2 kg and 21.5 ± 0.5 months of age were randomly assigned to receive either a diet containing sugar cane silage, ground corn cob, ground sorghum grain, soybean meal, protected urea, and a mineral/vitamin premix (control diet) or a diet in which ground sorghum grain was partially replaced with crude glycerol (crude glycerol diet), as described in Table 1.

After the first randomization by initial BW and age to each diet group (control or crude glycerol), animals were again randomly assigned according to the type of housing. Twelve animals were housed in individual pens and 16 animals were housed in four collective pens (four animals per pen).

The individual pens measured 2 meters wide by 5 meters long (10 m²/animal) with provision of a 5 m² shade by a zinc roof, whereas the collective pens measured 5 meters wide by 10 meters long (12.5 m²/animal) with no provision of shade. The volumetric capacity of feeders in the individual and collective pens was 0.35 and 1.05 m³, respectively. The length of the feed bunk in each collective pen was 3.8 meters, allowing 0.95 m³/animal.

There were six drinkers alongside the twelve individual pens (one drinker for two animals) with a capacity of 240 liters. There were two drinkers that supplied water for the four collective pens (one drinker in the border between two pens) with a capacity of 380 liters. Drinkers in the individual pens were under shading and in the collective pens were exposed to the sun. All drinkers had automatic floats that allowed a continuous water flow.

Table 1- Ingredients and nutritional composition of the experimental diets¹.

Ingredients % of DM	Control diet	Crude glycerol diet
Sugar cane silage	31.5	31.5
Ground corncob	20.0	17.0
Ground sorghum grain	33.5	18.5
Crude glycerol	-	18.0
Soybean meal	12.0	15.0
Protected urea (PROTE-N [®])	0.5	0.5
Limestone (CaCO ₃)	0.8	0.8
Dicalcium phosphate (CaHPO ₄)	0.3	0.3
NaCl	0.2	0.2
Mineral/vitamin premix ²	1.0	1.2
Nutritional composition		
DM ³ , %	63.3 ± 3.4	61.4 ± 2.9
CP, % of DM	12.2 ± 0.5	12.1 ± 1.0
NDF, % of DM	34.1 ± 0.4	30.0 ± 0.9
Ash, % of DM	4.9 ± 0.7	6.1 ± 0.9
GE, (1000 kcal/kg of DM)	4.2 ± 0.1	4.1 ± 0.1

¹ Mean analysis for composite samples (n = 6) and associated standard deviations; ² 18% Ca, 2.5 g/kg P, 17 g/kg Mg, 26.7 g/kg S, 66.7 g/kg Na, 25.2 mg/kg Cu, 416 mg/kg Co, 416 mg/kg Cu, 490 mg/kg Fe, 25.2 mg/kg I, 832 mg/kg Mn, 7 mg/kg Se, 2,000 mg/kg Zn, 833.5 mg/kg Monenzin, 83,200 IU/kg vitamin A, 10,400 IU/kg vitamin D, 240 IU/kg vitamin E; ³ Dry matter; Crude protein; Neutral detergent fiber; Gross energy.

Crude glycerol (80.5% glycerol, 11.9% moisture, 5.2% NaCl, and 50 mg of methanol/kg of glycerol; donated by "ADM do Brasil LTDA") was included at 15% of the ration dry matter (DM) as a partial replacement for ground sorghum grain throughout the entire study (Table 1). Soybean meal was added in a greater quantity (three percentage units) in the crude glycerol diet to adjust the crude protein (CP) levels of diets (Table 1).

Animals were fed once daily between 05:00 to 07:00 am in amounts that ensured *ad libitum* intake (10 to 15% oforts). During the ensiling of sugar cane urea was added (1 kg/100 kg; green matter basis) to reduce ethanol production during the fermentation process (Bravo-Martins et al., 2006; Castro Neto et al., 2008).

The experimental diets were formulated to contain similar levels of energy and CP, and balanced to meet the NRC (2000) guidelines for beef cattle in a feedlot system with an expected weight gain of 1.8 kg/day. All experimental protocols were approved by the IF Goiano Ethical Committee in the Use of Animals (decision # 4/2015).

Sample collection and analysis

Sugar cane silage samples were collected weekly and dried in a forced-air circulation oven for 72 hours at 65°C for DM analysis (AOAC, 2000) with the objective to maintain the nutritional value of the diets constant during the entire experiment. Samples of diets were collected every two weeks and stored frozen at -4°C. Soon after the end of the experiment, samples were thawed at room temperature, merged to form one composite sample of each treatment/14 days, and dried in a forced-air circulation oven for 72 hours at 65°C for DM analysis (AOAC, 2000). Subsequently, samples of diets were ground using a Willey mill to pass a 1-mm screen, and analyzed for CP, ash (AOAC, 2000), neutral detergent fiber (NDF) (Goering and Van Soest, 1970), and for gross energy (GE) in a Parr 6200[®] calorimeter.

Feed refusals were weighed daily and dry matter intake (DMI) was determined by difference between feed offered and feed refused. Body weight was recorded on days 1, 13, 27, 41, 55, 69, and 83 days after the beginning of the experiment after a twelve-hour solid fasting.

Feed intake pattern was determined on days 14, 28, 42, 56, 70, and 84 days after the beginning of the study in three moments relative to feed delivery (4, 10, and 24 hours). In each of the times indicated, the remaining feed from each individual or collective pen was briefly removed, weighed, and a 1-kg subsample was obtained (including from feed delivery) for DM (AOAC,

2000), NDF (Goering and Van Soest, 1970) and GE (Parr 6200[®] calorimeter) analyses. Feed intake pattern was calculated as follows: DMI 0-4 hours: kg of DM offered during feed delivery minus kg of DM remaining at 4 hours post-feeding; DMI 4-10 hours: kg of DM offered during feed delivery minus kg of DM remaining at 4 hours post-feeding minus kg of DM remaining at 10 hours post-feeding; DMI 10-24 hours: kg of DM offered during feed delivery minus kg of DM remaining at 10 hours post-feeding minus kg of DM remaining at 24 hours post-feeding.

Animals were slaughtered on October 6th of 2015 in Mineiros, Goiás State. Prior to transportation to the slaughterhouse, animals were weighed after a twelve-hour solid fasting and slaughtered following the procedures and normal flow of the abattoir. After hide removal and evisceration, carcasses were weighed to determine the hot carcass weight. Dressing percentage was calculated as the proportion between hot carcass weight and BW prior to slaughter.

Data analysis

The experimental design utilized was completely randomized in a factorial scheme 2 × 2 (two energy sources and two types of housing). The data were analyzed using the open system "R" (R Core Team, 2014) in a mixed model of double repeated measurements in time, considering energy source (sorghum or crude glycerol) and type of housing (individual or collective pens) as fixed effects, and animal as random. The structure of covariance that best fitted to the model was chosen according to the lowest Bayesian Information Criterion.

The model accounted for the effects of energy source (s), housing (h), days of experiment (d), times post-feeding (t; only for feed intake pattern measurements), energy source × days of experiment, energy source × times post-feeding, energy source × days of experiment × times post-feeding,

housing × days of experiment, housing × times post-feeding, housing × days of experiment × times post-feeding, energy source × housing, energy source × housing × days of experiment, energy source × housing × times post-feeding, days of experiment × times post-feeding, and energy source × housing × days of experiment × times post-feeding, according to the following equation:

$$y_{ijklm} = \mu + s_i + h_j + d_k + t_l + sd_{ik} + st_{il} + sdt_{lki} + hd_{jk} + ht_{jl} + hdt_{ljk} + sh_{ij} + shd_{ijk} + sht_{lji} + dt_{kl} + shdt_{ijkl} + e_{ijklm};$$

where y = independent variable, μ = mean, and e = experimental error.

When a fixed effect was significant ($P \leq 0.05$), means were compared using the Tukey test. Values are reported as least square means and associated standard errors of means (SEM).

RESULTS

Animals fed crude glycerol had daily DMI similar ($P > 0.05$) to animals fed the control diet (Table 2) regardless the type of housing (individual or collective pens). An energy source × days of experiment effect ($P < 0.05$) was observed due to a reduced DMI for the crude glycerol-fed animals housed in the individual pens during the first 14 days of the trial (Table 2).

When the daily DMI data were aggregated as seven-day means, animals fed crude glycerol decreased ($P < 0.05$) the DMI on days 7 (10.27 versus 8.81 ± 0.83 kg/day, control versus crude glycerol, respectively) and 14 (11.02 versus 9.46 ± 0.83 kg/day, control versus crude glycerol, respectively) days of the experiment (Figure 1, panel A). The same pattern occurred when the DMI was expressed as a % of BW and g/kg of metabolic weight, in which crude glycerol-fed animals reduced ($P < 0.05$) the DMI on days 7 (2.27 versus $2.00 \pm 0.15\%$ of

BW; 104.67 versus 91.58 ± 7.21 g/kg $BW^{0.75}$, control versus crude glycerol, respectively) and 14 of the trial (2.27 versus $2.08 \pm 0.15\%$ of $BW^{0.75}$, control versus crude glycerol, respectively), as shown in Figure 1 (panels B and C, respectively).

Animals fed crude glycerol had a more efficient ($P < 0.05$) feed conversion ratio (FCR) on days 63 (10.91 versus 6.08 ± 1.49 kg DM/kg gain, control versus crude glycerol, respectively) and 42 (10.94 versus 6.08 ± 1.49 kg DM/kg gain, control versus crude glycerol, respectively) of the study (Figure 1, panel D). Contrarily, as still shown in Figure 1 (panel D), crude glycerol-fed animals had a less efficient ($P < 0.05$) FCR on days 63 (6.83 versus 9.68 ± 1.49 kg DM/kg gain, control versus crude glycerol, respectively) and 70 (7.05 versus 9.83 ± 1.49 kg DM/kg gain, control versus crude glycerol, respectively) of the experiment.

Table 2- Effect of the substitution of sorghum grain with crude glycerol on the dry matter intake (DMI).

DMI	Control diet	Crude glycerol	SEM ²	P-value		
				Energy	Days ³	Energy × days
Individual pens						
kg/day	11.34	11.28	0.58	0.95	<0.05	<0.05
BW%	2.21	2.22	0.10	0.97	<0.05	<0.05
g/kg $BW^{0.75}$	105.34	105.38	4.76	0.99	<0.05	<0.05
FCR ¹ (kg DM/kg gain)	8.51	7.31	0.52	0.13	<0.05	<0.05
Collective pens						
kg/day	45.48	45.15	2.83	0.94	<0.05	0.69
BW%	2.24	2.17	0.10	0.69	<0.05	0.59
g/kg $BW^{0.75}$	150.10	146.38	7.5	0.76	<0.05	0.60
FCR (kg DM/kg gain)	9.56	7.89	1.18	0.42	<0.05	0.99

¹Feed conversion ratio, ²Standard error of means, ³Days when DMI was determined (1-84).

Feed intake pattern was not changed ($P > 0.05$) by the energy source in animals housed in individual and collective pens when the intake was expressed as kg of DM and kg of GE (Table 3). Nevertheless, crude glycerol-fed animals decreased ($P < 0.05$) the mean NDF intake pattern both in individual (1.44 versus 1.17 ± 0.05 kg,

control versus crude glycerol, respectively) and collective (5.72 versus 4.76 ± 0.25 kg, control versus crude glycerol, respectively) housing (Table 3). Furthermore, an energy source \times hours post-feeding response ($P < 0.05$) was detected for feed intake pattern measured in kg of DM (individual housing), kg of NDF (individual and collective housing) and kg of GE (individual housing) (Table 3). Animals fed crude glycerol and housed in individual pens decreased ($P < 0.05$) DM (5.62 versus 4.22 ± 0.31 kg, control versus crude glycerol, respectively), NDF (1.81 versus 1.21 ± 0.10 kg, control versus crude glycerol, respectively), and GE (23.37 versus 17.16 ± 1.31 kg, control versus crude glycerol, respectively) intake during the first four hours after fresh feed was delivered (Figure 2; panels A, B, and C, respectively). Likewise, crude glycerol fed animals housed in collective pens reduced ($P < 0.05$) the amount of NDF ingested from 0-4 (5.80 versus 4.71 ± 0.44 kg, control versus crude glycerol, respectively) and 4-10 (5.73 versus 4.26 ± 0.44 kg, control versus crude glycerol, respectively) hours post-feeding (Figure 2, panel D).

Weight gain, hot carcass weight and dressing percentage were not altered ($P > 0.05$) by the substitution of sorghum grain by crude glycerol in the diet (Table 4).

DISCUSSION

There is a decent number of studies in the literature reporting that crude glycerol can effectively replace traditional energy sources in beef cattle diets without negative effects on animal performance, such as barley (Mach *et al.*, 2009; Bartoň *et al.*, 2013; Egea *et al.*, 2014), ground corn (Moriel *et al.*, 2011; Ramos and Kerley, 2012; Leão *et al.*, 2013; Eiras *et al.*, 2014; van Cleef *et al.*, 2014; Fávares *et al.*, 2015) and

steam-flaked corn (Buttrey *et al.*, 2015). However, to the best of our knowledge, there is no information in the literature about Nelore animals fed crude glycerol as a primary feed ingredient of the diet (15% of the total DM ration) that substituted sorghum grain, which brings a novel aspect in the present study. Several experiments demonstrated that DMI was not influenced by substituting different energy sources with crude glycerol in the diet (Egea *et al.*, 2014; Eiras *et al.*, 2014; van Cleef *et al.*, 2014; Buttrey *et al.*, 2015; Fávares *et al.*, 2015), which corroborate the findings in the present work. Conversely, a linear reduction in DMI was observed when dry-rolled corn-based diets were replaced by increasing levels of crude glycerol (0 to 15% of the total DM ration) fed to finishing steers (Hales *et al.*, 2012). Likewise, increasing crude glycerol to 4, 8, 12, and 16% of the total DM ration fed to finishing beef heifers as a substitute for steam-flaked corn resulted in a linear decrease in DMI, but no changes in DMI occurred when crude glycerol was fed at either 0 or 2% of the diet (Parsons *et al.*, 2009).

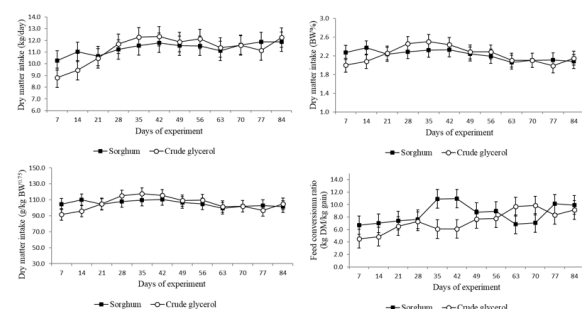


Figure 1- Effect of energy source (sorghum or crude glycerol) \times days of experiment (1-84) on dry matter intake (DMI) expressed as kg/day (panel A), BW% (panel B), $\text{g/kg BW}^{0.75}$ (panel C), and on feed conversion ratio (panel D) of 28 Nelore males housed in individual pens.

Table 3- Effect of the substitution of sorghum grain with crude glycerol on feed intake pattern.

Intake, IP ¹	Energy source	Intervals post-feeding (hours)			SEM ²	P-value					
		0-4	4-10	10-24		Energy	Days ⁶	Hours ⁷	Energy x days	Energy x hours	Energy x days x hours
DM, kg	Sorghum	5.62	4.13	3.84	0.31	0.12	<0.05	<0.05	0.68	<0.05	0.12
	Glycerol	4.22	3.59	4.39							
NDF ³ , kg	Sorghum	1.81	1.29	1.22	0.10	<0.05	<0.05	<0.05	<0.05	<0.05	0.05
	Glycerol	1.21	1.04	1.27							
GE ⁴ , kg	Sorghum	23.37	16.99	16.04	1.31	0.06	<0.05	<0.05	0.47	<0.05	0.14
	Glycerol	17.17	14.47	18.00							
Intake, CP ⁴											
DM, kg	Sorghum	17.11	18.30	18.57	1.48	0.28	<0.05	<0.05	0.99	0.09	0.08
	Glycerol	13.09	14.85	21.66							
NDF, kg	Sorghum	5.80	5.73	5.64	0.45	<0.05	0.25	0.06	0.55	<0.05	0.40
	Glycerol	3.72	4.27	6.31							
GE, kg	Sorghum	70.49	76.46	75.59	1.48	0.20	<0.05	<0.05	0.98	0.09	0.06
	Glycerol	52.32	61.26	87.07							

¹Individual pens, ²Neutral detergent fiber, ³Gross energy, ⁴Collective pens, ⁵Standard error of means, ⁶Days when feed intake pattern was determined (14, 28, 42, 56, 70, and 84), ⁷Hours post-feeding when feed intake pattern was determined (4, 10, and 24).

The level of contaminants (mostly salts and methanol) contained in crude glycerol has been suggested to be a possible reason for the DMI reduction in beef and dairy cattle. Both Parsons et al. (2009) and Hales et al. (2015) did not report the composition of crude glycerol in their studies, however, DeFrain et al. (2004) reported a DMI reduction in dairy cows supplemented with crude glycerol that contained 1.3% of methanol during the prepartum period, which is much higher than the standard recommended by FDA (2006) (15 mg of methanol/kg of glycerol or 0.015% methanol). It is important to underline that crude glycerol in the present work contained 50 mg of methanol/kg of glycerol, which is a third of the FDA (2006) recommendation.

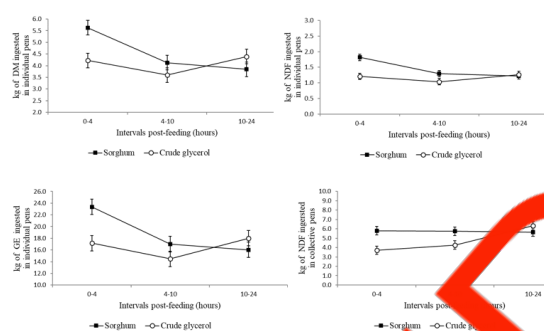


Figure 2- Effect of energy source (sorghum or crude glycerol) x intervals post feeding (0-4, 4-10 and 10-24 hours) on feed intake pattern expressed as kg of DM ingested (panel A), kg of NDF ingested (panel B), kg of CP ingested (panel C), and kg of NDF ingested (panel D) of 28 Nelore males housed in individual (panels A, B, and C) or collective (panel D) pens.

The DMI decrease expressed as kg/d, BW% and g/kg of metabolic weight during the first 14 days of the experiment may be explained by a lack of adaptation of rumen microbes to a new ingredient in the diet that animals had never experienced before, although Jobson and Mann (1961) indicated that regardless of the diet, glycerol is fermented by *Selenomonas ruminantium*, which is a basic component of the rumen flora. A 14-day period of adaptation is standard in feedlot experiments, but it is difficult to state whether or not rumen microbes in crude glycerol-fed animals were completely adapted without the determination of species in the rumen flora.

The fact that crude glycerol-fed animals decreased the NDF intake pattern in different intervals of the day (0-4, 4-10, and 10-24 hours after fresh feed was delivered) was unexpected, considering that glycerol has been reported to coat the fibrous fraction of the ration and increase the preference for long (>19 mm) and medium (<19, >8 mm) particles of the diet (Carvalho et al., 2012) due to its viscous property and sweet-tasting (Ayoub and Abdullah,

2012). Besides, there is clear evidence that DMI in ruminants is regulated by a neurological response according to the taste of feeds (Provenza, 1995), which would favor sweet-tasting diet ingredients, such as crude glycerol.

In contrast, fluctuations in the NDF content between diets may partly elucidate the NDF intake pattern differences. The NDF concentration in the control diet was 13.66% higher than the crude glycerol diet (34.1 versus 30%, control versus crude glycerol, respectively, Table 1), but NDF intake pattern in the control diet was 23.07 and 20.16% greater than the crude glycerol diet in individual and collective housing, respectively. Therefore, the NDF variation between diets cannot entirely explain such differences. Yet, corroborating the findings in this study, Leão *et al.* (2012), Fávares *et al.* (2011) and Hales *et al.* (2015) reported a linear reduction in NDF intake with increasing levels of crude glycerol in the diet (0 to 24, 0 to 20, and 0 to 15% of the total DM ration, respectively), but none of these authors explained the reasons for the NDF intake reduction.

Table 4- Effect of the substitution of sorghum grain with crude glycerol on weight gain.

Item	Energy source		P-value						
	Sorghum grain	Crude glycerol	SEM ⁴	Energy	Housing	Days ⁵	Energy × days	Energy × housing	Energy × housing × days
Birth weight									
Day 1	437.3	437.9							
Day 13	468.1	465.9							
Day 27									
Day 41	514.8	520.0	11.8	0.81	0.75	<0.05	0.39	0.62	0.64
Day 55	531.5	542.0							
Day 69	549.5	559.0							
Day 83	571.7	581.7							
HCW ² , kg	308.3	316.7	6.0	0.33	0.81	-	-	0.23	-
DP ³ , %	54.0	54.5	0.5	0.48	0.36	-	-	0.16	-

¹Body weight, ²Hot carcass weight, ³Dressing percentage, ⁴Standard error of means, ⁵Days when BW was recorded (1, 13, 27, 41, 55, 69, and 83)

The energy source × hours post-feeding effect in which crude glycerol-fed animals reduced the intake of DM (individual pens), NDF (individual and collective pens) and GE (individual pens) mostly within four hours post-feeding is difficult to explain without passage rate and metabolic parameters, but can also be partly elucidated by a lack of adaptation of rumen microbes to a new ingredient in the diet.

Although animals fed crude glycerol decreased the overall DMI during the first 14 days of the experiment, reduced the NDF intake pattern and decreased DM, NDF and GE intake within four hours post-feeding, crude glycerol-fed animals had similar BW compared with animals fed the control diet. In addition, there was a tendency for a more efficient FCR for crude glycerol-fed animals, which is a very important measurement for the commercial application of this diet.

One of the explanations why the substitution of traditional energy sources (grains and cereals) with crude glycerol did not affect the performance of beef cattle in the present study and many others is based on the evidence that glycerol increased the molar proportion of propionate at the expense of acetate (Rémond *et al.*, 1993; Wang *et al.*, 2009; Carvalho *et al.*, 2011; Ramos and Kerley, 2012; Bartoň *et al.*, 2013) or can be directly absorbed by the rumen epithelium (Rémond *et al.*, 1993). In both scenarios glycerol can act as a gluconeogenic precursor in the liver and consequently crude glycerol-fed animals may have been benefited from an enhanced energy status. This argument may explain the decreased DM and GE intake during the first four hours post-feeding. Further research is necessary with the assessment of physiological measures to corroborate whether or not beef cattle have a more constant feed intake pattern due to an enhanced energy status by crude glycerol feeding.

A second reason can be found in studies with swine, where it was reported that crude glycerol contained similar digestible and metabolizable energy as corn grain (Lammers et al., 2008; Kerr et al., 2009).

Collectively, the data reported in this study clearly indicate that the substitution of sorghum grain with crude glycerol is safe and brings no adverse effects on the performance of Nelore animals finished in feedlots. It is important to be cautious to the methanol concentration in crude glycerol to avoid any adverse effect.

The recent growth of biodiesel production has led to increased stocks of crude glycerol with a subsequent price reduction, which has ranged from US\$ 0.04/kg to US\$ 0.33/kg (Ayoub and Abdullah, 2012). Given that sorghum grain contains 88.13% DM, 9.67% CP, 15.31% NDF, and 2.94% ether extract on a DM basis (NRC, 2000), the crude glycerol breakeven substitution price on a DM basis can be calculated by accounting for the value of CP, NDF and ether extract that are lost when sorghum grain is removed from the diet and replaced by crude glycerol. By this method, the crude glycerol breakeven substitution price = sorghum grain price – (sorghum grain price × 0.0967) – (sorghum grain price × 0.1531) – (sorghum grain price × 0.0294). The cost of utilizing crude glycerol in beef cattle diets should also account for the cost of freight from the biodiesel production plant to the feedlot and also for investment with storage infrastructure due to crude glycerol viscosity.

CONCLUSIONS

Crude glycerol can safely substitute sorghum grain at 15% of the total DM ration in diets fed to beef cattle with no detrimental effects on animal performance. Other factors such as price competitiveness, cost of freight

and storage infrastructure should account for the utilization of crude glycerol in rations fed to beef cattle finished in feedlots.

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REFERENCES

- AGÊNCIA NACIONAL DO PETRÓLEO, GÁS NATURAL E BIOCOMBUSTÍVEIS (ANP). Produção e fornecimento de biocombustíveis. Rio de Janeiro, 2017. Available at <http://www.anp.gov.br/wwwanp/images/DADOS_ESTADISTICOS/Producao_bio_diesel/Producao-de-Biodiesel-m3.xls>. Accessed in September 10th, 2017.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, Maryland, 2000. 1298p.
- AYOUB, M.; ABDULLAH, A.Z. Critical review on the current scenario and significance of crude glycerol resulting from biodiesel industry towards more sustainable renewable energy industry. Renewable and Sustainable Energy Reviews, v.16, n.5, p.2671-2686, 2012.
- BARTOŇ, L.; BUREŠ, D.; HOMOLKA, P. et al. Effects of long-term feeding of

crude glycerine on performance, carcass traits, meat quality, and blood and rumen metabolites of finishing bulls. *Livestock Science*, v.155, n.1, p.53-59, 2013.

BRAVO-MARTINS, C.E.C.; CARNEIRO, H.; CASTRO-GÓMEZ, R.J.H. et al. Chemical and microbiological evaluation of ensiled sugar cane with different additives. *Brazilian Journal of Microbiology*, v.37, n.4, p.499-504, 2006.

BUTTREY, E.K.; LUEBBE, M.K.; McCOLLUM III, F.T. et al. Effects of glycerin concentration in steam-flaked corn-based diets with supplemental yellow grease on performance and carcass characteristics of finishing beef steers. *Journal of Animal Science*, v.93, n.7, p.3698-3703, 2015.

CARVALHO, E.R.; SCHMELZ-ROBERTS, N.S.; WHITE, H.M. et al. Replacing corn with glycerol in diets for transition dairy cows. *Journal of Dairy Science*, v.94, n.2, p.908-916, 2011.

CARVALHO, E.R.; SCHMELZ-ROBERTS, N.S.; WHITE, H.M. et al. Feeding behaviors of transition dairy cows fed glycerol as a replacement of corn. *Journal of Dairy Science*, v.95, n.12, p.7214-7224, 2012.

CASTRO NETO, G.; MOLINA, L.R.; GONÇALVES, L.S. et al. Parâmetros de fermentação de silagem de cana-de-açúcar submetidas a diferentes tratamentos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, v.60, n.5, p.1450-1456, 2008.

van CLEEF, F.H.C.B.; EZEQUIEL, J.M.B.; D'AVILA, A.P. et al. Crude glycerol in diets for feedlot Nelore cattle. *Revista Brasileira de Zootecnia*, v.43, n.2, p.86-91, 2014.

DUBRAIN, J.M.; HIPPEN, A.R.; KALSCHER, K.F. et al. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. *Journal of Dairy Science*, v.87, n.12, p.4195-4206, 2004.

DEPARTMENT OF ECONOMIC AND SOCIAL AFFAIRS OF THE UNITED NATIONS. *World Population Prospects. The 2017 Revision*, 2017. Available at <https://esa.un.org/unpd/wpp/Publications/Files/WPP2017_KeyFindings.pdf>. Accessed in September 19th, 2017.

EGEA, M.; LINARES, M.B.; GARRIDO, M.D. et al. Crude glycerol inclusion in Limousin bull diets: animal performance, carcass characteristics and meat quality. *Meat Science*, v.98, n.4, p.673-678, 2014.

EIRAS, C.E.; BARROS, L.P.; MARQUES, A. et al. Glycerine levels in the diets of crossbred bulls finished in feedlot: apparent digestibility, feed intake and animal performance. *Animal Feed Science and Technology*, v.197, p.222-226, 2014.

FILVARO, V.R.; EZEQUIEL, J.M.B.; D'AVILA, A.P. et al. Glycerin in cattle feed: intake, digestibility, and ruminal and blood parameters. *Semina: Ciências Agrárias*, v.36, n.3, p.1495-1506, 2015.

GOERING, H.K.; van SOEST, P.J. Forage fiber analysis (Apparatus, Reagents, Procedures and Some Applications). *Agricultural Handbook n° 379*. Agricultural Research Service - USDA, Washington, DC, USA, 1970. 20p.

HALES, K.E.; FOOTE, A.P.; BROWN-BRANDL, T.M. et al. Effects of dietary glycerin inclusion at 0, 5, 10, and 15 percent of dry matter on energy metabolism and nutrient balance in finishing beef steers. *Journal of Animal Science*, v.93, n.1, p.348-356, 2015.

HOBSON, P.N.; MANN, S.O. The isolation of glycerol-fermenting and lipolytic bacteria from the rumen of the sheep. *Journal of General Microbiology*, v.25, p.227-240, 1961.

KERR, B.J.; WEBER, T.E.; DOZIER III, W.A. et al. Digestible and metabolizable energy content of crude glycerol originating from different sources in nursery pigs. *Journal of Animal Science*, v.87, n.12, p.4042-4049, 2009.

- LAMMERS, P.J.; KERR, B.J.; WEBER, T.E. et al. Digestible and metabolizable energy of crude glycerol for growing pigs. *Journal of Animal Science*, v.86, n.3, p.602-608, 2008.
- LEÃO, J.P.; NEIVA, J.N.M.; RESTLE, J. et al. Consumo e desempenho de bovinos de aptidão leiteira em confinamento alimentados com glicerol. *Ciência Animal Brasileira*, v.13, n.4, p.421-428, 2012.
- MACH, N.; BACH, A.; DEVANT, M. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. *Journal of Animal Science*, v.87, n.2, p.632-638, 2009.
- MORIEL, P.; NAYIGIHUGU, V.; CAPPELLOZZA, B.I. et al. Camelina meal and crude glycerin as feed supplements for developing replacement beef heifers. *Journal of Animal Science*, v.89, n.12, p.4314-4324, 2011.
- NATIONAL RESEARCH COUNCIL - NRC. Nutrient requirements of beef cattle. 7th ed. Washington, DC: National Academy Press, USA, 2000. 25p.
- PARSONS, G.L.; SHELOR, M.K.; DROUILLARD, S. Performance and carcass traits of finishing heifers fed crude glycerin. *Journal of Animal Science*, v.87, n.2, p.653-657, 2009.
- PROVENZA, F.D. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management*, v.48, n.1, p.2-17, 1995.
- R CORE TEAM. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, 2014. Available at <<http://www.R-project.org>> Accessed in October 5th, 2017.
- RAMOS, M.H.; KERLEY, M.S. Effect of dietary crude glycerol level on ruminal fermentation in continuous culture and growth performance of beef calves. *Journal of Animal Science*, v.90, n.3, p.892-899, 2012.
- RÉMOND, B.; SOUDAY, E.; JOUANY, J.P. In vitro and in vivo fermentation of glycerol by rumen microbes. *Animal Feed Science and Technology*, v.41, n.2, p.121-132, 1993.
- THOMPSON, J.C.; HE, B.F. Characterization of crude glycerol for biodiesel production from multiple feedstocks. *Applied Engineering in Agriculture*, v.22, n.2, p.161-203, 2006.
- U.S. FOOD & DRUG ADMINISTRATION - FDA. Code of Federal Regulation, 21 CFR 582.1320, Title 21, vol. 6, 21CFR582.1320, 2006. Available at <http://edocket.access.gpo.gov/cfr_2002/quarter/21cfr582.1320.htm> Accessed in September 15th, 2017.
- WAN, J.; LIU, Q.; HUO, W.J. et al. Effects of glycerol on rumen fermentation, urinary excretion of purine derivatives and feed digestibility in steers. *Livestock Science*, v.121, n.1, p.15-20, 2009.