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Crude glycerin in diets for feedlot Nellore cattle

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ABSTRACT - Two studies were conducted to evaluate the effects of crude glycerin on feed intake, performance, carcass characteristics, and total digestibility of Nellore bulls. In experiment 1, cattle (n = 30) were fed a control diet without crude glycerin and diets containing 7.5, 15, 22.5, and 30% crude glycerin, for 103 d. Animals were harvested and data of carcass characteristics were collected. In experiment 2, a digestibility trial was performed using indigestible acid detergent fiber (iADF) as internal marker, and five rumen-cannulated steers. Both experiments were conducted as a randomized complete block design and data were analyzed using mixed procedures. In experiment 1 no differences were observed among treatments on dry matter intake, and performance variables. Regarding carcass characteristics, no effect was observed, except for carcass fat estimates, which were greater in treatments with crude glycerin. In experiment 2, crude glycerin promoted a decrease in digestibility of fibrous fractions NDF and HEM, and increased digestibility of crude protein by 6%. Although it caused negative effect on digestibility of fibrous fraction of diets, crude glycerin can be a good energy source for Nellore bulls, since no losses are observed on performance and carcass characteristics when animals are fed up to 30% of this by-product.

Key Words: beef cattle, by-products, carcass, digestion, glycerol

Introduction

An irreversible consequence of the increasing world production of biodiesel is the surplus production of its by-products, such as oilseeds meals, cakes, and mainly crude glycerin. In order to minimize the costs of feeding, studies have been conducted to evaluate the use of these by-products as livestock feed ingredients, generating, thus, an environmentally friendly destination for agribusiness waste.

Crude glycerin, the most common form of glycerin used in animal nutrition, is a product obtained from oil processing for the chemical industry and biodiesel production, and for every nine parts of biodiesel produced, one part of glycerin is formed (Dasari et al., 2005). It is basically composed of glycerol and varying amounts of water and other impurities such as alcohols and catalysts.

Pure glycerin has a wide range of applications such as emulsifiers, fabric softener, plasticizer, stabilizer and wetting agent in breads, ice cream and tobacco. Besides these, glycerin can be used in skin lotions, mouthwashes, in many cosmetic and pharmaceutical preparations, and as a means of protection for freezing blood cells, sperm and other tissues.

The use of crude glycerin (with impurities), obtained directly from the production of biodiesel, is a major challenge, since this by-product cannot be directly used in the cosmetic or food industries. Furthermore, the effective use or conversion of glycerin into specific products reduces the cost of production of biodiesel, meat and milk.

This by-product has been intensively tested as ingredient in diets for poultry (Dozier et al., 2008; Jung and Batal, 2011), swine (Kerr et al., 2009; Schieck et al, 2010), beef cattle (Pyatt et al., 2007; Parsons et al., 2009; Mach et al., 2009), dairy cattle (Chung et al., 2007; Donkin, 2008), sheep (Gunn et al., 2010a; Gunn et al., 2010b), and other species, such as goats (Hampy, 2007), rabbits (Iñigo et al., 2011) and fish (Li et al., 2010).

Crude glycerin has the potential of replacing corn in ruminant diets (Donkin, 2008) since the glycerol can be converted to glucose in the liver of ruminants, providing energy for cellular metabolism (Goff and Horst, 2001).

Most research is being conducted using glycerin in beef cattle fed high concentrate diets (Parsons et al., 2009). However, previous studies show that glycerin, even at low concentrations, has the potential to decrease fiber digestibility (Parsons and Drouillard, 2010). Therefore, this study has the objective to examine the effects of crude glycerin on feed intake, performance, carcass characteristics and total tract apparent digestibility of feedlot Nellore bulls.

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Material and Methods

The first experiment was carried out at the experimental feedlot of the Animal Science Department of Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal City, Brazil. Thirty Nellore bulls (227.7±23.8 kg BW and 18 months old) were housed in individual concrete-surfaced, semi-roofed pens (12 m^2), and distributed in a complete randomized block design, according to the initial weight, with five treatments and six replicates. The pens had individual feed bunks and water tanks.

The total experimental period lasted 103 d, of which 21 d were spent on adaptation and 82 d on the experimental recording period. Upon arrival, all cattle were offered free access to corn silage and water before processing. Two days after arrival, animals were vaccinated, dewormed, supplemented with vitamins A, D, E and K, and tagged. Cattle were gradually adapted to a 70% concentrate diets (21 days).

Five experimental diets containing the same amount of protein (12.2% CP/DM) and energy (2.5 Mcal ME/DM) were formulated according to NRC (1996) and fed *ad libitum* to animals twice daily at a concentrate:roughage ratio of 70:30, and were named G0, G7.5, G15, G22.5 and G30, which corresponded to the treatments containing inclusion of 0, 7.5, 15, 22.5 and 30% of crude glycerin on a dietary dry matter basis (Table 1).

Table 1 - Percentage of feed ingredients and nutrient composition of experimental diets (DM basis)

Item	Treatments				
	G0	G7.5	G15	G22.5	G30
Ingredients					
Corn silage	30.00	30.00	30.00	30.00	30.00
Corn grain	35.00	25.50	18.00	12.50	5.00
Soybean hulls	19.20	18.05	14.55	8.90	5.45
Sunflower meal	14.60	17.80	21.30	24.90	28.40
Crude glycerin	0.00	7.50	15.00	22.50	30.00
Salt (NaCl)	0.50	0.50	0.50	0.50	0.50
Limestone	0.70	0.65	0.55	0.70	0.65
Dicalcium phosphate	0.00	0.00	0.10	0.00	0.00
Nutrients					
CP (% DM)	12.20	12.21	12.22	12.20	12.21
RDP (% CP)[1]	67.41	70.74	74.11	77.39	80.79
ME (Mcal/kg DM)	2.53	2.52	2.52	2.53	2.52
EE (%)	2.90	2.58	2.31	2.09	1.81
NDF (%)	40.83	40.07	38.11	35.01	33.08
ADF (%)	25.41	25.56	24.71	22.89	22.06
HEM (%)	15.42	14.51	13.41	12.12	11.02
Calcium (%)	0.56	0.56	0.55	0.57	0.55
Phosphorus (%)	0.32	0.32	0.35	0.34	0.34

Treatments: G0 - no addition of crude glycerin; G7.5 - addition of 7.5% crude glycerin; G15 - addition of 15% crude glycerin; G22.5 - addition of 22.5% crude glycerin; G30 - addition of 30% crude glycerin.
CP - crude protein; RDP - rumen-degradable protein; ME - metabolizable energy; EE - ether extract; NDF - neutral detergent fiber; ADF - acid detergent fiber; HEM - hemicellulose.
[1] According to Valadares Filho et al. (2010).

The crude glycerin used in this experiment was composed of 95% dry matter, 86% glycerol, 6% salts, and less than 0.01% methanol. The roughage used was corn silage and the concentrate contained ground corn grain, soybean hulls, sunflower meal, and one of the four concentrations of crude glycerin.

The concentrate and corn silage were weighed and mixed with crude glycerin at the moment of feeding. Before subsequent feeding, 10% of orts of each animal were sampled in order to monitor dry matter daily intake.

Samples of feed and orts were combined at the end of experiment and analyzed for dry matter, ether extract and crude protein according to AOAC (1995), and neutral detergent fiber and acid detergent fiber according to Goering and Van Soest (1970), in order to calculate nutrient intakes.

Animals were weighed upon arrival, at the end of adaptation period, and every 28 days of the experimental period, for monitoring the evolution of weight gain. At the end of the experimental period, the average daily gain as well as feed efficiency were calculated. The animals were taken to a slaughterhouse where they were stunned by brain concussion, using an air-injection captive bolt stun gun and then were bled from the jugular vein.

At slaughter, hot carcass weight was measured so dressing percentage could be calculated, dividing its result by the slaughter weight. After cooling for 24 hours at 4 °C, carcasses were weighed again (cold carcass weight). After this, samples were taken from the left half carcass, between the 9th, 10th and 11th ribs, and divided into muscle, fat and bone, whose weights were used for the indirect determination of carcass composition (Hankins and Howe, 1946).

The *longissimus* muscle was exposed between the 12th and 13th ribs for its area to be measured by using the square grid method. The backfat thickness was measured in the third quarter of the muscle from the spine, perpendicular to the *longissimus* muscle by using a precision scale. The carcass pH was measured by introducing the pH meter probe vertically to a depth of approximately 5 cm in the *longissimus* muscle at the last rib. The carcasses were divided into primal commercial cuts: hindquarter (tenderloin, top and bottom sirloin, outer, upper and lower round, rump and back shank), forequarter (chunk, brisket, neck, hump, front shank and five ribs), and spare ribs (flank and short ribs).

The second experiment was carried out at Animal Unit of Digestive and Metabolic Studies of the Animal Science Department of Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal City, Brazil.

The internal marker indigestible acid detergent fiber (iADF) was used to estimate dry matter, organic matter, crude protein, ether extract, neutral detergent fiber and acid detergent fiber digestibilities. For that, feed, orts, and feces were sampled from the Study 1 (performance) animals in the first three days of the sixth week of the performance trial. The samples were pre-dried in an oven at 55 °C for 72 h and formed composite samples per animal. Fecal samples were collected at the time of defecation, representing 24 h during three consecutive days.

The percentage of iADF was quantified after 264 h of *in situ* incubation (Casali et al., 2008), using 100 µm nylon bags (14 × 7 cm) containing 4.5 g of sample, on a dry matter basis. The bags were attached to a chain and placed into the rumen of five rumen-cannulated Nellore steers (400 kg BW and 24 months old). The animals were adapted to the experimental diets (Study 1), for 14 d prior to the incubation.

After the incubation period, the bags were manually washed in fresh water until the residue of rumen content was removed. Then, the bags were pre-dried in an oven at 55 °C for 72 h and weighed so the dry matter disappearance could be calculated. After that, the residual samples were removed from the bags and ground (1 mm particles) to be analyzed for NFD and ADF (Goering and Van Soest, 1970).

For both experiments, data were analyzed as a completely randomized block design by using the MIXED procedure of SAS (Statistical Analysis System, version 9.1). Animal was the experimental unit, and model effects included block (random effect) and treatments (fixed effect), according to the equation: $Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$, in which Y_{ij} = observed measurement, μ = overall mean; τ_i = inclusion level of crude glycerin (i = 0, 7.5, 15, 22.5, 30%); β_j = effect of block (j = 1 to 6); and ε_{ij} = experimental error. Contrasts were used to determine the linear and quadratic effects of glycerin, and 0% glycerin × glycerin treatment. In addition, means of treatments were obtained with the LSMEANS option. Contrasts were considered significant when $P \leq 0.05$.

Results and Discussion

Increasing the levels of crude glycerin to Nellore bulls had no effect ($P>0.05$) on dry matter intake. Compared with results of studies using highly-processed and highly-digestible ingredients, the components of the diets used in present study present rapidly-degradable protein in the rumen and/or fibers of low and high quality, such as sunflower meal and soybean hulls. As the glycerin is rapidly used in the rumen (6 h with addition of 15 to 25%, according to Bergner et al., 1995), the result of this association may have been synergistic, enhancing the use of glycerin as well as the other ingredients, thus not interfering with dry matter intake by animals. D'Aurea (2010), studying the effect of inclusion of 0, 10 or 20% crude glycerin in the diet of Nellore finishing heifers, observed increase in dry matter intake when 10% crude glycerin (9.7 kg) were included, and the treatments 20% crude glycerin and control (without glycerin) did not differ (8.7 and 8.0 kg, respectively). Interestingly, the experimental diets were formulated by the authors with ingredients from the same source as used in the present study. Gunn et al. (2010a) worked with lambs fed up to 20% glycerin in a by-products-based diets and observed no differences in dry matter intake.

The result observed in this study disagrees with Parsons et al. (2009), who observed a decrease in dry matter intake in beef heifers receiving finishing flaked-corn-based diets with up to 16% crude glycerin in the dietary dry matter. Likewise, Schneider et al. (2010) observed a decrease in DMI when they fed (n = 295) 0, 0.5, or 2% crude glycerin in flaked corn-based diets to heifers.

There were no differences among treatments ($P>0.10$) for average daily gain, final body weight, and feed conversion ratio (Table 2), probably due to the maintenance of dry matter intake, although increases in feed efficiency related to decrease in dry matter intake and maintaining the gains have been reported.

Despite the large numerical difference in the observed performance parameters (Table 2), it was not possible to statistically prove ($P>0.05$) these changes caused by the inclusion of glycerin. This was possibly due to the wide variation of data and the relatively low number of replicates used (six per treatment). Parsons et al. (2009), working with crossbred heifers fed 0, 2, 4, 8, 12, or 16% crude glycerin in diets containing steam flaked corn, observed quadratic effect on average daily gain, in which the intermediate treatments with 2, 4 and 8 % inclusion of glycerin presented higher values (1.34, 1.29 and 1.25 kg, respectively). These authors also identified quadratic effect ($P<0.05$) on feed efficiency, in which the treatment with 2% glycerin was the best. In contrast, there was a decrease in final weight when the animals were fed the highest doses of glycerin.

In another study, Schneider (2010) provided 0, 4 or 8% crude glycerin for heifers fed diets containing 60% corn silage and observed a linear effect ($P<0.01$) on feed efficiency, which was improved with increasing addition of crude glycerin in the diet. However, Gunn et al. (2010b), including up to 45% glycerin in sheep diets, observed linear decrease ($P<0.01$) in feed efficiency and average daily gain, which made the authors to conclude that the maximum value of addition of glycerin to sheep diets should be 15%, on a dry matter basis. Ramos and Kerley (2012) included up to 20% glycerin in diets rich in forage or concentrate for calves and observed no changes in dry matter intake or in animal performance parameters, agreeing with the results obtained in this study.

There was no effect (P>0.10) of addition of crude glycerin in most of carcass traits evaluated (Table 3). The carcass traits are directly or indirectly related to DM intake. As no variation was observed in intake or performance, the lack of effect on these variables is justified.

Carcass composition may vary with the composition of the diets, as evidenced by the change observed in the estimated percentage of carcass fat (P<0.05). This fact is probably related to the increase in propionate production promoted by the inclusion of crude glycerin in the diets (Wang et al., 2009; Ramos and Kerley, 2012), providing greater available energy to the animals. The glycerol absorbed on the rumen wall can be converted to glucose in the liver. The enzyme glycerol kinase converts the glycerol + ATP to glycerol-3-phosphate + ADP at the triose phosphate level, directing the glycerol towards gluconeogenesis (Krehbiel, 2008). The glycerol absorbed after the rumen can be converted to glucose by the same biochemical pathway.

In agreement with the results found in the present research, Mach et al. (2009) fed Holstein steers up to 12% glycerin on concentrate dry matter and found no effect on carcass characteristics and meat quality. D'Aurea (2010) reported the same observation when including up to 20% crude glycerin in the diet of Nellore heifers.

Parsons et al. (2009) fed crossbred heifers up to 16% crude glycerin and observed quadratic effect (P<0.01) on hot carcass weight, with an increase of 8.1, 5.1 and 3.3 kg when 2, 4 and 8% glycerin were added, respectively. The LM area and fat thickness decreased linearly (P<0.05) as the percentage of glycerin was increased. These authors expected more fat deposition (as observed in the present study) and higher marbling score due to the assumption that the glycerol is rapidly converted to propionate in the liver.

As a result of the second experiment, addition of crude glycerin had no effect on apparent digestibility of DM, OM, EE, and ADF, estimated by indigestible acid detergent fiber marker (Table 4). However, linear increase was observed (P>0.01) on crude protein digestibility and, when the analysis of the contrast 0 × GLY was performed, regardless of the concentration of crude glycerin used, the digestibility of crude protein was higher for treatments in

Table 2 - Dry matter intake and performance of Nellore bulls (n = 30) fed diets containing up to 30% crude glycerin for 103 days

Item	Crude glycerin (%)					Contrast			SEM
	0	7.5	15	22.5	30	Linear	Quadratic	0 × GLY[1]	
Initial body weight, kg	279.5	280.5	270.5	279.3	278.5	0.82	0.37	0.65	4.46
Final body weight, kg	413.9	427.6	423.1	427.3	403.5	0.67	0.28	0.72	15.84
Dry matter intake, kg/d	8.96	7.81	8.49	8.75	7.79	0.27	0.97	0.10	0.38
Average daily gain, kg/d	1.54	1.69	1.75	1.70	1.44	0.68	0.12	0.57	0.15
Feed conversion, kg/kg	0.19	0.22	0.21	0.20	0.19	0.45	0.31	0.74	0.02

SEM - standard error of the mean.
*P<0.05; **P<0.01.
[1] Control × glycerin treatment.

Table 3 - Carcass characteristics of Nellore bulls (n = 30) fed diets containing up to 30% crude glycerin for 103 days

Item	Crude glycerin (%)					Contrast			SEM
	0	7.5	15	22.5	30	Linear	Quadratic	0 × GLY[1]	
Hot carcass weight, kg	219.5	225.2	227.5	231.9	214.0	0.86	0.13	0.55	7.59
Cold carcass weight, kg	217.8	223.8	226.4	230.9	213.3	0.95	0.13	0.51	7.67
Dressing percentage, %	53.05	52.70	53.87	54.32	53.03	0.38	0.23	0.50	0.56
Longissimus muscle area, cm^2	65.17	63.33	64.83	63.67	64.50	0.93	0.83	0.77	3.32
Backfat thickness, mm	5.33	5.67	5.67	7.67	4.33	0.24	0.12	0.88	0.80
Carcass pH	6.12	6.03	6.06	6.03	6.19	0.74	0.34	0.73	0.12
Commercial cuts									
Hindquarter, %	51.42	53.62	54.08	54.80	50.81	0.92	0.11	0.32	1.64
Forequarter, %	45.92	45.92	46.77	42.92	44.80	0.55	0.92	0.79	2.64
Spare ribs, %	13.47	14.12	13.75	14.62	12.67	0.67	0.19	0.71	0.74
Carcass composition									
Bone, %	20.11	19.30	18.72	19.06	19.73	0.62	0.11	0.21	0.62
Muscle, %	56.34	54.49	53.53	56.29	54.63	0.73	0.48	0.36	1.54
Body fat, %	22.79	25.79	27.37	24.32	25.05	0.37	0.12	0.02	1.05

SEM - standard error of the mean.
*P<0.05; **P<0.01.
[1] Control × glycerin treatment.

Table 4 - Digestibility of diet dry matter, and nutrients in cattle fed crude glycerin, obtained by using the internal marker iADF

Item	Crude glycerin (%)					Contrast			SEM
	0	7.5	15	22.5	30	Linear	Quadratic	0 × GLY[1]	
Dry matter	63.8	64.6	64.0	65.7	65.8	0.17	0.84	0.34	1.16
Organic matter	68.9	69.6	68.7	68.7	70.0	0.90	0.88	0.66	0.60
Crude protein	56.1	60.1	61.2	61.9	62.2	<0.01	0.18	<0.01	1.48
Neutral detergent fiber	51.5	54.9	50.1	46.0	45.3	<0.01	0.27	0.21	1.74
Acid detergent fiber	29.8	34.4	30.9	32.7	32.3	0.44	0.34	0.07	1.30
Ether extract	86.0	85.7	86.9	85.2	87.5	0.50	0.61	0.81	1.15
Hemicelluloses	54.4	54.7	54.4	50.1	49.3	<0.01	0.12	0.06	1.02

SEM - standard error of the mean.
*P<0.05; **P<0.01.
[1] Control × glycerin treatment.

which glycerin was added. The sunflower meal was used to adjust the concentration of crude protein in the diets, so as not to include a new ingredient in the formulation. Thus, the concentration of rumen degradable protein was linearly increased, and it probably increased the protein digestibility both by the greater use by ruminal microorganisms, and by the greater absorption at the intestines.

Neutral detergent fiber digestibility was linearly affected (P<0.05), in which the value observed for the treatment with inclusion of 30% crude glycerin showed a decrease of 11.38% compared with the control treatment. The same effect was observed (P<0.01) when the digestibility of hemicelluloses was evaluated, which presented a decrease of 5.37% in the digestibility, comparing the 30%-crude-glycerin treatment with the control treatment. Although there was a decrease in the digestibility of NDF, and HEM fractions, the low concentrations of these fractions in diets (33-44% and 11-15%, respectively) were not sufficient to affect DM intake and performance.

The mechanism of action of glycerin on the populations of fibrolytic bacteria, and consequently on digestibility and degradability of fibrous fraction of diets, is still unclear, and may be related to three main factors: 1 - the formation of an environment unfavorable to the multiplication of these bacteria, such as osmolarity and pH; 2- the physical protection of fibrous particles preventing the adhesion of bacteria; and 3- preference or competition for another substrate, in this case, glycerol.

D'Aurea (2010), adding 0, 10 or 20% crude glycerin in the diet of Nellore heifers, observed decrease in apparent digestibility of NDF and hemicellulose, and the values of the treatment with 20% glycerin (42.3 and 54.2%, respectively) were similar to those obtained in the present study. Parsons and Drouillard (2010), including 0, 2 or 4% crude glycerin in diets of heifers, also observed a linear decrease effect on NDF digestibility. Abo El-Nor et al. (2010) included 3.6, 7.2, and 10.8% glycerin in diets with 60% alfalfa hay for Holstein cows and found no effect on the *in vitro* dry matter digestibility, but when 7.2 or 10.8% glycerin were added, the *in vitro* NDF digestibility decreased (P<0.05), and the populations of *B. fibrisolvens* and *S. ruminantium* (fibrolytic bacteria) decreased.

Schneider (2010), working with crossbred heifers fed 0, 4, or 8% crude glycerin, observed a linear decrease (P<0.01) in the digestibility of DM, OM and NDF. Lage et al. (2010) observed quadratic effect (P<0.05) on NDF digestibility, obtaining 38.44% of digestibility when 7.3% glycerin was included in the diet of sheep. In another digestibility study, Wang et al. (2009) provided 0, 100, 200, and 300 g of glycerin to cattle and observed a quadratic effect on digestibility of DM, OM, CP, NDF and ADF, in which the values found for the intermediate concentrations were better when compared with treatment with higher amounts of glycerin and with the control treatment.

In contrast to the previous studies cited, Hampy (2007) provided 5 and 10% glycerin to meat goats and observed no effect on digestibility of either DM or NDF. When an *in vitro* experiment was performed, using higher concentrations of glycerin (up to 20%), no deleterious effect on NDF digestibility was reported (Hampy, 2007), proving the large range of results related to crude glycerin as an energy ingredient, probably due to the large difference in its composition and nutritive value.

Another important aspect related to the use of crude glycerin in diets for finishing cattle would be the possibility of this by-product replacing energy ingredients, especially corn, whose price, at certain times of the year, reaches very high prices, making its use in feedlot diets uneconomical.

Conclusions

Despite the negative effect on digestibility of the dietary fibrous fraction, crude glycerin can be a good energy source in diets for Nellore bulls, since no difference is observed on performance and carcass characteristics of animals fed up to 30% of this by-product.

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