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Effects of partial or total replacement of corn cracked grain with high concentrations of crude glycerin on rumen metabolism of crossbred sheep

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ABSTRACT

Crude glycerin, a by-product of the biodiesel industry, has been used as a strategic ingredient in intensive ruminant production systems, mainly in substitution of starch-rich ingredients. The present study was performed to evaluate the effects of the inclusion of up to 30% of crude glycerin in diets for feedlot sheep, on ruminal parameters, such as pH, NH_3-N and volatile fatty acids concentrations, *in situ* degradability, as well as *in vitro* greenhouse gas production and *in vitro* digestibility. Eight ruminally-cannulated male Santa Inês × Dorper sheep (64.5 ± 8.5 kg) were distributed in a replicated 4 × 4 Latin square design. The experimental diets contained 0, 10, 20 or 30% of crude glycerin and were labeled as G0, G10, G20 and G30, respectively. The crude glycerin totally replaced the corn cracked grain in treatment G30. The inclusion of crude glycerin in the diets tended to promote a quadratic effect in DMI, with greater values observed for treatments G10 and G20. Crude glycerin tended to increase the ruminal pH and NH_3-N, but linearly reduced the total molar concentration of VFA, acetic, butyric, isobutyric and isovaleric acids. Treatments linearly increased *in vitro* DM digestibility of diets and linearly reduced NDF digestibility. The inclusion of crude glycerin in the diets linearly decreased the *in vitro* total gas and CO_2 production (mL/g degraded) and tented to reduce CH_4 (mL/g degraded). A linear increase of soluble fraction in water ("a") of the diets was observed with the increasing inclusion of crude glycerin. The insoluble but potentially degradable fraction ("b") of DM and NDF of the diets were linearly decreased and increased, respectively. The potential ruminal degradation of the diets was markedly and linearly increased with the increasing inclusion of the by-product. The replacement of corn cracked grain by crude glycerin (up to 30% DM) changes rumen fermentation parameters, decreasing VFA production, *in vitro* total gas production and CH_4. Additionally, the potential and effective degradation as well as *in vitro* DM digestibility of diets are improved while fiber digestibility is impaired.

1. Introduction

The use of agroindustry by-products as animal feed is becoming increasingly common throughout the world. The high price of conventional ingredients, such as corn and soybean, has led meat producers to look for alternative ingredients, which usually have a lower purchase cost, but require further understanding of their nutritional value and acceptability (van Cleef et al., 2014).

Crude glycerin, a by-product of the biodiesel industry, is a new well-documented ingredient, which has been used as a strategic ingredient in intensive ruminant production systems, mainly in substitution of starch-rich ingredients, such as corn (Donkin et al., 2009; Carvalho et al., 2015; Almeida et al., 2017; van Cleef et al., 2017). The current annual production of biodiesel in the world is around 34.5 billion liters OECD/FAO (2016). The Brazil is the second largest biodiesel producer in the world, with 3.8 billion liters, in 2016 (United States lead with 5.5 billion liters), generating around 420 million liters of crude glycerin in 2016 (ANP, 2017), that could be used to feed livestock.

This by-product is mainly composed of glycerol, which is an energetic compound of great assimilation by rumen microorganisms and with extensive metabolism in the liver (Abo El-Nor et al., 2010). In the rumen, glycerol is rapidly metabolized by microorganisms to form short chain fatty acids, mainly propionate and butyrate (Donkin, 2008; AbuGhazaleh et al., 2011). The glycerol disappears almost entirely from the rumen in the first 24 h (Trabue et al., 2007), but can also be directly absorbed by the epithelium of the digestive system and act as a gluconeogenic substrate in the liver (Krehbiel, 2008).

The fermentation of glycerin may promote better stability to the

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rumen environment compared with starch-rich ingredients, mainly by reducing lactic acidosis as a consequence of the increase in the population of lactate-consuming bacteria and undue fermentation (Krueger et al., 2010). However, they may have a detrimental effect on the growth of structural carbohydrate fermenting bacteria (Roger et al., 1992; AbuGhazaleh et al., 2011), resulting in reduced fiber digestibility and methane production (Shin et al., 2012; van Cleef et al., 2015).

Nonetheless, recent studies have shown that the inclusion of high levels of crude glycerin does not impair the consumption, performance or carcass characteristics of feedlot sheep (Gunn et al., 2010a; Gunn et al., 2010b; Gomes et al., 2011). Therefore, it is essential to study high inclusions of crude glycerin in total substitution to corn, with evaluations of rumen fermentation characteristics, to establish an adequate level for a healthier rumen environment.

Therefore, the objective of this study was to evaluate the effects of the inclusion of up to 30% of crude glycerin (on DM basis) in diets for feedlot crossbred sheep, on ruminal parameters, such as pH, NH_3-N, VFA concentrations, *in situ* DM and NDF degradabilities, as well as *in vitro* greenhouse gas production and DM and nutrients *in vitro* digestibility.

2. Materials and methods

The study was conducted at the Animal Unit of Digestive and Metabolic Studies from the Department of Animal Science of São Paulo State University (Unesp), Jaboticabal, São Paulo, Brazil. The São Paulo State University Institutional Animal Care and Use Committee approved all experimental protocols adopted in the current study (approval number: 06329/14).

2.1. Animals, diets and experimental design

Eight ruminally-cannulated crossbred uncastrated male Santa Inês × Dorper lambs (64.5 ± 8.5 kg and approximately 18 months old) were distributed in a replicated 4 × 4 Latin square arrangement of treatments, according to initial body weight. The animals were housed in individual semi-roofed, concrete-surfaced pens (2.5 m^2), with individual feed bunks and waterers, and received the experimental diets for 21-d periods, including 14 d of adaptation, followed by 7 d of sample collection.

The experimental diets contained 0, 10, 20 or 30% of crude glycerin (on DM basis) and were formulated to be isonitrogenous (17.7% CP/DM) and isoenergetic (2.7–2.8 Kcal ME/kg DM) to supply the requirements of a 20–30 kg lamb with moderate growth for daily gains of 200 g, according to NRC (2007), and with a roughage:concentrate ratio of 40:60. The dietary treatments were labeled as: G0 (control treatment, containing no crude glycerin), G10 (containing 10% crude glycerin in diet DM), G20 (containing 20% crude glycerin in diet DM), and G30 (containing 30% crude glycerin in diet DM). The crude glycerin totally replaced the corn cracked grain in treatment G30 (Table 1).

The crude glycerin used in this trial contained 95% DM, 83% glycerol,1.1% CP, 6% salts, 4.8% other compounds and less than 0.01% methanol. The concentrate and corn silage were weighed and mixed with crude glycerin at the moment of feeding (0700 and 1900 h), delivering 50% of total mixed ration in each meal. Before subsequent feeding, orts were weighed and approximately10% of each animal were sampled to determine DM to adjust feed delivery and to monitor daily dry matter intake.

2.2. Dry matter intake, rumen pH, ammonia nitrogen, and VFA profiles

The concentrate and corn silage were weighed and mixed with crude glycerin at the moment of feeding, delivering 50% of total in each meal. Before subsequent feeding, samples of orts of each animal were collected to monitor dry matter daily intake.

Rumen fluid samples were collected on d 15 of each experimental period, at 0, 2, 4, 6, 8, 10, and 12 h after feeding to measure pH, and evaluate ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) concentrations. Approximately 500 g of ruminal contents of each animal were collected from the dorsal and ventral rumen sites, and strained through four layers of cheesecloth to separate liquid and solid phases. The pH was measured immediately after rumen fluid sampling using a digital pH meter (model Digimed DM-20; Digicrom Analítica Ltda, São Paulo, SP Brazil), and NH_3-N concentrations was determined using a micro-Kjeldhal apparatus (model TE-0364; Tecnal Equip. para Laboratórios, Piracicaba, SP, Brazil), with 5 mL of KOH 2N, and a distillation flux of 2 mL/min. Samples were centrifuged at 3000 × *g* for 20 min, and the supernatant was used to determine NH_3-N. The distilled sample was dropped in 10 mL boric acid solution (2%), and then titrated with HCl 0.005N.

Approximately 2.0 mL of rumen fluid was centrifuged twice (12,000 × *g* for 15 min at 4 °C (Sorvall Superspeed RC2-B, Newton, CT, USA)) with formic acid 98–100% (Merck KGaA). After centrifugation, approximately 0.5 mL of supernatant was transferred to chromatographic vials. The concentration of VFA was determined by injecting 0.5 µL of sample in a gas chromatograph (TRACE 1300, Thermo Scientific, MA, USA) equipped with a HP-FFAP capillary column (19091F-112; 25 m; 0.320 mm; 0.50 µm; J&W Agilent Technologies Inc.; Palo Alto, CA, USA). The carrier gas was helium at a flow rate of 1 mL/min. The oven temperature program was 1 min at 60 °C, followed by an increase to 200 °C at a rate of 5 °C/min. The injector temperature was 270 °C, and the detector temperature was 300 °C. The sample was injected into a split/splitless system (split ratio 1:10). The calibration curve was made using chromatographic standards (Chem Service, West Chester, PA, USA) of acetic acid (99.5%; CAS 64-19-97), propionic acid (99%; CAS 79-09-4), isobutyric acid (99%; CAS 79-31-2), butyric acid (98.7%; CAS 107-92-6), isovaleric acid (99%; CAS 503-74-2), and valeric acid (99%; CAS 109-52-4).

Table 1
Ingredient and chemical composition of diets containing 0 (G0), 10 (G10), 20 (G20) or 30% (G30) of crude glycerin.

Item	Treatments			
	G0	G10	G20	G30
Ingredient composition (%)				
Corn silage	40.0	40.0	40.0	40.0
Corn cracked grain	30.0	20.0	10.0	0.0
Soybean hulls	7.8	7.2	6.3	4.5
Soybean meal	20.6	21.0	21.6	23.1
Urea	0.6	0.9	1.1	1.3
Crude glycerin	0.0	10.0	20.0	30.0
Mineral/vitamin premix[a]	0.5	0.5	0.5	0.5
Limestone	0.5	0.5	0.5	0.5
Bicalcium phosphate	0.0	0.0	0.0	0.2
Nutrient composition				
DM, %	65.8	66.1	66.4	66.6
CP, %	17.7	17.7	17.7	17.7
ME, Mcal/kg	2.8	2.8	2.7	2.7
EE, %	3.0	2.7	2.3	2.0
aNDF, %	34.8	33.0	31.1	28.7
ADF, %	19.2	18.5	17.7	16.5
Ca, %	0.5	0.5	0.5	0.5
P, %	0.3	0.3	0.3	0.3

[a] Composition per kg: P (75 g), Ca (223 g), S (10 g), Zn (3 g), Na (60 g), Co (20 mg), I (40 mg), Se (24 mg), F (750 mg), Mg (5 g), Mn (1.8 g), Fe (402 mg), Vit A (312,500 UI), Vit D (50,000 UI), Vit E (437 UI).

2.3. In vitro total tract digestibility

In vitro digestibility of DM and NDF was assessed using the methodology proposed by Holden (1999). On d 21 of each experimental period, approximately 1 kg of ruminal content were collected from each