Livestock Science 192 (2016) 17–24

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effect of crude glycerine in supplement on the intake, rumen fermentation, and microbial profile of Nellore steers grazing tropical grass ☆

Elias San Vito [a,*], Juliana D. Messana [a], Pablo S. Castagnino [a], Yury T. Granja-Salcedo [a], Erick E. Dallantonia [a], Telma T. Berchielli [a,b]

[a] Department of Animal Science, College of Agrarian and Veterinarian Sciences (FCAV), Univ Estadual Paulista – UNESP, Jaboticabal, São Paulo, Brazil
[b] INCT/CA – UFV – Department of Animal Science, Viçosa, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 16 January 2016
Received in revised form
15 August 2016
Accepted 16 August 2016

Keywords:
Bacteria
Degradation
Forage
Glycerol
Protozoa

ABSTRACT

The objective of this study was to evaluate the effect of crude glycerine (CG) inclusion in feed supplement (0, 70, 140, 210 and 280 g/kg DM basis of supplement) on the forage intake and digestibility, ruminal fermentation parameters, kinetics of fibre degradation, and rumen microbial profile of Nellore steers grazing on tropical grass. Ten ruminally cannulated Nellore steers [490 kg ± 47 body weight (BW)] were used in a replicated 5 × 5 Latin square design with 14-d periods. Steers were individually supplemented at the rate of 300 g/100 kg of BW. Inclusion of CG in the supplement did not affect ($P > 0.05$) dry matter intake, apparent total tract digestibility, ruminal pH ($P=0.784$) or total ruminal VFA ($P=0.291$), but linearly decreased the NH_3-N concentration ($P=0.021$). The inclusion of CG in the supplement linearly increased ($P < 0.001$) the molar proportions of butyrate and valerate; linearly decreased the acetate ($P=0.007$) concentration, thus reducing the acetate to propionate ratio ($P < 0.001$); and did not affect the molar proportions of propionate and isobutyrate. Inclusion of GC had a quadratic effect ($P=0.010$) on the *in situ* potential degradable fraction of NDF and rate of fibre degradation ($P=0.006$). Addition of CG linearly decreased the number of protozoa of the genera *Entodinium* ($P=0.015$) and *Isotricha* ($P=0.058$) and the relative proportions of *Ruminococcus albus* ($P=0.047$) and *Ruminococcus flavefaciens* in the rumen ($P=0.036$), but did not affect *Fibrobacter succinogenes* ($P=0.420$) or the methanogens ($P=0.150$). The inclusion of CG in the supplement up to 280 g/kg DM altered ruminal fermentation and negatively affected *in situ* fibre degradation and the gram-positive cellulolytic bacterial population, but did not affect intake and apparent total tract digestibility for Nellore steers grazing tropical grass.

1. Introduction

Global biofuel policies have led to the shifting of large volumes of food crops to bioethanol and biodiesel production, resulting in price increases in several agricultural commodities, including corn (Popp et al., 2014). Not only is corn a stabile human food; it is an important feed grain for livestock. Its high market price is driving livestock producers to search for alternative feed sources.

Crude glycerine (CG), a by-product of biodiesel production, has become an attractive alternative to grain as an energy source in ruminant diets (Bartoň et al., 2013; Donkin et al., 2009; Mach et al., 2009). Crude glycerine is preferentially converted to propionate in the rumen (Wang et al., 2009), absorbed directly by ruminal epithelium or go directly to the small intestine and then converted to glucose in the liver (Krehbiel, 2008). In addition, *in vitro* studies where glycerol was added to the substrate have found a linear increase in propionate to the detriment of acetic acid production (Avila-Stagno et al., 2013; Castagnino et al., 2015) and reduction in the acetate to propionate ratio (Avila et al., 2011; Bergner et al., 1995). Since propionate fermentation has been suggested as a means of reducing methane emissions, inclusion of CG in the diets of ruminants may reduce emissions, particularly when added to high-fibre diets (Rémond et al., 1993).

However, the nutritional value and ruminal fermentation parameters of inclusion of CG in forage-based diets in the literature are still insufficient. Moreover, there is a shortage of information regarding the effect of CG on microbial communities, especially those related to methane production. Only *in vitro* studies have produced results on the effect of glycerol inclusion on fibre degradation activity mediated by rumen microorganisms

☆ Crude glycerine for Nellore steers grazing tropical grass.

* Correspondence to: Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, São Paulo State University, Rod. Professor Paulo Donato Castellane, km 5, Rural, Jaboticabal, São Paulo CEP: 14884-900, Brazil.
E-mail address: esanvito@zootecnista.com.br (E. San Vito).

http://dx.doi.org/10.1016/j.livsci.2016.08.011
1871-1413/

Table 1
Proportion of ingredients and analysed chemical composition of supplements and forage of *Brachiaria brizantha* 'Xaraés'.

	Crude glycerine in the supplement, g/kg DM					
	0	70	140	210	280	Forage[a]
Ingredient composition[b], g/kg						
Corn	500.0	410.0	332.0	253.0	175.0	–
Crude glycerine	0.0	70.0	140.0	210.0	280.0	–
Corn gluten	0.0	20.0	28.0	37.0	45.0	–
Soybean meal	420.0	420.0	420.0	420.0	420.0	–
Urea/ammonium sulphate	30.0	30.0	30.0	30.0	30.0	–
Commercial premix[c]	50.0	50.0	50.0	50.0	50.0	–
Chemical composition, g/kg DM						
Dry matter	917.0	915.7	914.0	912.4	910.8	890.8 ± 28.3
Organic matter	916.2	913.3	910.4	907.6	904.8	918.5 ± 4.48
Crude protein	370.5	370.9	370.6	370.4	370.1	180.9 ± 23.9
NDF[d]	140.4	130.1	120.0	100.8	90.7	570.5 ± 28.7
Ether extract	30.8	30.5	30.2	20.9	20.6	14.4 ± 2.9
Non-fibrous carbohydrates[e]	400.7	410.6	430.0	440.4	450.9	138.9 ± 24.3
Crude energy, Mcal/kg of DM	4.11	4.12	4.11	4.11	4.10	4.09 ± 0.06

[a] Average and standard deviation of the mean of samples obtained by the technique of simulated grazing during five periods.
[b] Chemical composition g/kg of DM=Corn – DM: 905.8, OM: 981.6, CP: 119.5, NDF: 185.3, EE: 58.8, Crude energy (CE): 4.36 Mcal; Crude glycerine – DM: 879.8, OM: 942.3, CP: 11.5, EE: 18.1, CE: 4.14 Mcal; Corn gluten – DM: 928.5, OM: 971.4, CP: 706.3, NDF: 84.7, EE: 38.2, CE: 5.66 Mcal; Soybean meal – DM: 917.5, OM: 928.8, CP: 550.5, NDF: 156.1, EE: 21.6, CE: 4.59 Mcal.
[c] Composition=Calcium: 210 g/kg; Phosphorus: 20 g/kg; Sulphur: 37 g/kg, Sodium: 80 g/kg; Copper: 490 mg/kg; Manganese: 1.424 mg/kg; Zinc: 1.830 mg/kg; Iodine: 36 mg/kg; Cobalt: 29 mg/kg; Selenium: 9 mg/kg; Fluorine (Max): 333 mg/kg.
[d] NDF: neutral detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash.
[e] Calculated as 1000−(crude protein+ether extract+ash+neutral detergent fibre).

(Abo El-Nor et al., 2010; Danielsson et al., 2014; Roger et al., 1992). We hypothesized that crude glycerine as an energy component could provide an alternative to partially replace corn in supplement of forage-based diets for grazing cattle, will increase propionic acid production and modify the ruminal microbial population without affecting the total apparent digestibility and dry matter intake.

Thus, the objective of the present study was to evaluate the effects of inclusion of crude glycerine (0, 70, 140, 210 and 280 g/kg DM of supplement) as a replacement for corn in the forage-based diet of grazing Nellore steers on intake and digestibility of DM and nutrients, rumen fermentation parameters, and the rumen microbial population.

2. Materials and methods

The protocol used in this experiment was in accordance with the Brazilian College of Animal Experimentation (COBEA – Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (CEBEA – Comissão de Ética e Bem Estar Animal) of the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista – Jaboticabal campus (protocol number 21,119/11).

2.1. Experimental procedures

The trial was conducted during the rainy season at a location (Brazil, 21°15′22″ south, 48°18′58″ west and 595 m above sea level) owned by the Univ Estadual Paulista (UNESP, Jaboticabal, SP, Brazil) from February to May 2012. The climate is classified as tropical rainy with dry winter (Köppen international system: Aw). During the experimental period, the average monthly precipitation was 26.9 mm, with an average maximum monthly temperature of 33.7 °C and an average minimum monthly temperature of 13.8 °C. Ten ruminally cannulated (rubber rumen cannula 4″, KEHL®, São Carlos, BR) Nellore steers [490.1 ± 47.8 kg body weight (BW)] at 25 ± 2 months of age were used. Animals were distributed in a replicated 5 × 5 Latin square arrangement to assess the impact of different concentrations of crude glycerine (CG) in the supplement on forage intake, apparent digestibility of DM, OM, CP and NDF, ruminal pH, NH_3-N, ruminal concentration of VFA, fibre degradation, and ruminal microbiology over five 14-d periods. Each period consisted of 10 d of adaptation to the supplement and 4 d of sampling. The animals were weighed and treated against endo- and ecto-parasites by administration of ivermectin (Ivomec, Merial, Paulínea, BR) at the beginning of the experiment, then allocated into five paddocks (two animals per paddock) of 0.25 ha each. The animals and treatment were rotated between paddock. Pasture area was established in 2011, with planting of *Brachiaria brizantha* 'Xaraés' grass. Nitrogen was applied to each paddock as urea at a rate of 200 kg/ha, divided into two applications during the study. The paddocks were fitted with smooth wire fencing, waterers (with free access for the animals), and a pair of individual feed bunks.

Steers were individually supplemented at the rate of 300 g/100 kg BW daily at 10:00 a.m. The experimental treatment consisted of 0, 70, 140, 210, and 280 g/kg dry matter (DM) inclusion of CG in the supplement in place of corn. Crude glycerine was added to the concentrate supplement before supplementation to animals using a vertical mixer with a 500 kg capacity equipped with a device that allowed irrigating the CG in the other ingredients forming a homogeneous mixture. Mixing time was 10 min.

Crude glycerine [870.98 g/kg DM; 50.72 g/kg ash; 10.15 g/kg crude protein (CP); 10.81 g/kg ether extract (EE); 800.34 g/kg glycerol; 0.3 g/kg methanol] was purchased from a soybean-oil-based biodiesel production company (ADM, Rondonópolis, Brazil). Supplemental concentrations of corn gluten were increased with increasing CG to maintain similar concentrations of CP in the DM. Ingredients were sampled every 15 d to determine their chemical composition (Table 1). Individual steers' BW was recorded at the beginning of each period, without a fasting period, for adjustment of the supplementation rate.

Forage height was randomly measured weekly at 20 points using a graduated stick in each paddock (Barthram, 1985). Forage mass measurements were taken using three samples per paddock in three sampling periods during the experiment. Samples were collected by clipping all forage within a 0.25-m^2 frame in each