THE EFFECT OF SOYBEAN MOLASSES ON *IN VITRO* RUMINAL FERMENTATION OF CORN SILAGE-BASED FEEDLOT FINISHING DIET

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Abstract: We evaluated the effects of increasing levels of soybean molasses (SM) as replacement of ground corn in feedlot corn silage-based diets on *in vitro* gas production. The ground corn was replaced by 0, 50, 100, and 150 g kg^{-1} of SM in the diets. Gas production (GP) kinetics were recorded using a commercial apparatus (Ankom^{RF} GP System). After 24 h, the incubation was stopped and the pH of the fluid was determined and filtrate used to determine *in vitro* dry matter (IVDMD) and organic matter digestibility (IVOMD). The levels of SM linearly increased gas production from 0 to 4 h (P<0.05). However, after 8 h (8-12 h and 12-16 h of incubation) there were negative linear effects (P = 0.03), and from 16 to 20 h a trend was verified (P = 0.097) with SM inclusion on GP. The addition of SM did not altered (P>0.05) total GP, and IVDMD and IVOMD, with average values of 190 mL g^{-1} DM, 593 g/kg and 623 g/kg. The level of 50 g kg^{-1} presented the greater pH (6.94) than others levels (quadratic effect; P = 0.08). The increasing levels of SM did not promoted effects on total GP and *in vitro* digestibility.

Keywords: gas production, *in vitro* digestibility, ruminant

Introduction

Mato Grosso State is the largest national producer of soybean. For that reason, there are numerous agribusinesses that use it as raw material. Consequently, harvesting and industrial processes generate several agricultural residues, which one of these is the soybean molasses. Soybean molasses is a viscous liquid of brown color and bittersweet flavor, free of alcohol, formed mainly by sugars of glucose, fructose, sucrose, raffinose, and stachyose (Chajuss, 2004). This by-product have a high generation and low comercial cost. Its incorrect disposal can cause several enviromental problems, and its use to ruminant feeding can minimize this problem. Therefore, the objective of the present work is to evaluate the effects of increasing levels of soybean molasses as replacement of ground corn in feedlot corn silage-based diets on *in vitro* gas production.

Materials and Methods

All procedures involving animals were approved by the Institutional Animal Care and Use Committee. Feed ingredients consisting of ground corn, cottonseed cake, soybean molasses (SM), soy hulls, urea and mineral-vitamin supplement were used to mix 4 treatments for an *in vitro* fermentation study. All substrates contained similar crude protein (CP) content (136 g CP kg^{-1} of dietary dry matter, DM) and a roughage:concentrate ratio of 150:850. The following treatments were evaluated: control – no SM inclusion in the diet (SM0); SM50 – 50 g SM kg^{-1}; SM100 – 100 g SM kg^{-1}; SM150 – 150 g SM kg^{-1}. The SM inclusion concentrations are described on a DM basis. The averaged composition (on a DM basis) of the diets were: 152 g/kg of cottonseed cake; 150 g/kg corn silage; 150 g/kg soy hulls; 20 g/kg mineral-vitamin and 8 g/kg urea. The ground corn (520, 470, 420, and 370 g kg^{-1}) was replaced by SM in the diets, respectively to SM0, SM50, SM100 and SM150. The SM was obtained from soybean industry (Caramurú Alimentos, Sorriso, MT; 607 g DM kg^{-1}) contained (DM basis): 89 g CP kg^{-1}; 286 g sucrose kg^{-1}; 178 g stachyose kg^{-1}; 45 g raffinose kg^{-1}; 38 g fructose kg^{-1}; 8 g glucose kg^{-1}.

Ruminal fluid was obtained from two ruminally cannulated Holstein steers (442 ± 27.6 kg of body weight). The steers were grazing Marandu palisadegrass (*Brachiaria brizantha*) pasture and received 4 kg/day of commercial concentrate provided twice daily (9h30 and 17h30) to establish a 500 g concentrate kg^{-1} diet based on ground corn, soybean meal, and cottonseed cake (160 g CP kg^{-1}; Fortuna Nutrição Animal, Nova Canaã do Norte, MT). The rumen fluid was collected from four quadrants of the rumen, hand-squeezed, poured into 2 thermal flasks preheated to 39 ± 0.5°C, and immediately transferred to the laboratory.

On the day of incubation, each bottle (310 ml) was filled with 1.00 ± 0.008 g of each diet (ground through 1-mm sieve), 100 ml of fermentation fluid (consisting of 80 ml of rumen fluid and 20 ml of buffer solution) keeping headspace of bottle with CO_2. The buffer solution was prepared 24 h before each ruminal fluid collection, according to McDougall (1948). Substrates were incubated for 24 h at 39 ± 0.5°C in 250-mL bottles, and gas pressure to determine gas production (GP) kinetics were recorded using a commercial apparatus (Ankom^{RF} GP System). Incubations were performed on 4 non-consecutive days. The experimental design was as follows: 4 incubation runs × 4 treatments × 2 replications (1 from each animal) plus 2 blank bottles per run (only rumen fluid and buffer solution), for a total of 40 bottles.

After 24 h of incubation, flasks were removed and immediately placed on ice to suppress fermentation. Bottles were then opened and the contents of each bottle were filtered under vacuum through glass crucibles (pore size 100 to 160 µm). The fluid pH was measured with a pH meter and the obtained fermentation residues were dried at 105°C overnight to estimate DM disappearance. Residue OM was determined as loss on ignition in a muffle furnace at 550°C for 4 h.

Statistical analyses were performed using the MIXED procedure of SAS 9.4 according to a completely randomized design including the fixed effect of treatment and the random effects of steers and incubation run. Orthogonal polynomial contrasts were used to evaluate linear, quadratic and cubic components of the response to incremental amounts of SM. Separations of means were determined to be significant at an α level ≤ 0.05 and tendencies at $P > 0.05$ and $P \leq 0.10$. No cubic effects were observed ($P > 0.10$), and then they were not presented.

Results and Discussion

As SM increased there was a positive linear effect (P<0.05) on gas production from 0 to 4 h. The GP varied of 29.3 to 39.3 mL g DM with the SM levels. This can be justified by the greater concentration of sugars rapidly fermentable in the SM, such as sucrose, fructose, and glucose. The rates of sugar disappearance in rumen fermentation can range from 0.19 to 0.69 h^{-1} (Henning et al., 1991). The GP from 4 to 8 hours was not affected by the SM levels (P $\geq$ 0.35), which averaged 38.4 mL g DM. However, after 8 h (8-12 h and 12-16 h of incubation) there were negative linear effects (P = 0.03) and from 16 to 20 h a trend was verified (P = 0.097) with SM inclusion on GP. Therefore, these greater GP production from 8 to 20 h of incubation can be due the starch fermentation, which is less fermentable (0.06 h^{-1}) than single sugars (Herrera-Saldana et al., 1990). Effects of SM was not observed during 20 to 24 h (P > 0,15).

The addition of SM did not altered (P>0.05) total GP, and *in vitro* DM and OM digestibility (IVDMD and IVOMD, respectively) with average values of 190 mL g^{-1} DM, 593 g/kg and 623 g/kg (Table 1). The fluid pH tended to show quadratic relationship (P = 0.077) with increasing levels of SM in the diets. The SM5 level presented the greater pH (6.94) than others levels.

Menke et al. (1979) proposed an equation to estimate metabolizable energy (ME; MJ kg^{-1} DM) considering the total GP and CP content are positively related with the EM of the diet. Therefore, as the diets had similar CP content and total GP, we can infer that diets with SM levels presented similar EM. Thus, it seems that SM can replace ground corn up to 150 g kg^{-1} without affect ruminal fermentation and ME in feedlot corn silage-based diets.

Conclusion

Soybean molasses did not promoted effects on total gas production and *in vitro* organic matter digestibility.

Table 1 – Effects of increasing levels of soybean molasses as replacement of ground corn in corn silage-based diets on *in vitro* gas production.

	Soybean molasses level (g kg^{-1} DM)					P-value	
Item	0	50	100	150	SEM	Linear	Quadratic
Gas production (mL g^{-1} DM)							
0-4 h	29,3	32,9	38,9	39,3	4,16	0,040	0,512
4-8 h	35,59	36,34	42,94	38,63	5,318	0,352	0,500
8-12 h	41,40	37,65	38,98	29,27	4,117	0,030	0,363
12-16 h	34,19	34,69	33,52	24,83	3,132	0,032	0,109
16-20 h	26,17	25,86	24,81	20,18	2,660	0,097	0,371
20-24 h	19,18	17,68	17,99	14,79	2,117	0,153	0,644
Total	193,74	185,50	198,13	180,86	19,244	0,606	0,673
pH	6,88	6,94	6,91	6,86	0,078	0,568	0,077
IVDMD (g/kg DM)	599	595	580	597	44.2	0,708	0,424
IVOMD (g/kg OM)	625	622	614	630	31.5	0,916	0,407

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