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Effect of starch level in supplement with or without oil source on diet and apparent digestibility, rumen fermentation and microbial population of Nellore steers grazing tropical grass

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ABSTRACT

In this study, we evaluated the effects of starch level in supplement, with/without whole soybean (oil source), on diet intake and apparent digestibility, rumen microbial population, and fermentation parameters in Nellore steers grazing *Brachiaria brizantha* cultivar Xaraés during the finishing phase. Eight ruminal cannulated Nellore steers (514 kg ± 30) were used in a replicate 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments. The diets used had different levels of supplemented starch [corn (high) or soybean hulls (SH: low)] with/without a source of oil (soybean grain). There were no interactions between starch level and oil in supplements on the intake of DM (% of body weight, $P = 0.602$; kg/d, $P = 0.703$), forage DM ($P = 0.630$), supplement DM ($P = 0.501$), OM ($P = 0.669$), CP ($P = 0.420$), aNDFom ($P = 0.380$), EE ($P = 0.199$) and GE ($P = 0.620$). However, intake and total apparent digestibility of DM, OM, and aNDFom ($P < 0.050$) decreased with oil supplementation, whereas the intake and digestibility of aNDFom were increased ($P < 0.010$) with low-starch supplementation. There were no interactions between starch supplement and oil source supplementation for pH ($P = 0.391$), NH_3-N ($P = 0.473$), and total volatile fatty acids ($P = 0.441$). The inclusion of an oil source in supplements decreased ($P < 0.010$) the acetate concentration in the rumen when compared with supplements without oil, independent of starch level. Supplementation with high-starch increased the numbers of *Entodinium* ($P < 0.010$), and total protozoa ($P < 0.010$). Furthermore, independently of starch level, the addition of an oil source decreased the population of *Dasytricha* ($P < 0.010$), *Polyplastron* ($P < 0.010$), and *Diploplastron* ($P = 0.040$). Supplementing animals with low-starch (SH) without oil resulted in a higher proportion of *Ruminococcus albus* ($P = 0.012$) compared with the other supplements. Moreover, low-starch (SH) supplement, with or without oil, decreased the relative population of *Selenomonas ruminantium* ($P = 0.003$). The addition of oil in supplements decreased the number of *Fibrobacter succinogenes* ($P < 0.001$), *Ruminococcus flavefasciens* ($P < 0.001$), and *Archeas* ($P < 0.001$), but increased *Anaerovibrio lipolytica* populations ($P < 0.001$). Oil supplement decreased intake, digestibility, acetate production, protozoan populations, and fibrolytic rumen bacteria. The use of soybean hulls without whole soybean supplementation may be effective in increasing the digestibility of CP and *R. albus* in finishing Nellore steers grazing *B. brizantha* during the dry season.

1. Introduction

The nutritional strategy to increase uptake of nitrogen by rumen microorganisms in pasture-based systems has focused on improving the energy supply to animals. Dietary energy promotes ammonia incorporation into microbial protein, and thereby increases the amino acid flow to the small intestine (Moorby et al., 2006).

Ammonia is the preferred nitrogen source of fibrolytic bacteria in the rumen, and supply of ruminal degradable fibre (e.g. pectin) may stabilize ruminal pH and increase ruminal ammonia utilization (Russell et al., 1992). Nevertheless, the rate of absorption of ammonia by ruminants suggests that energy availability, or lack of synchrony between energy and nitrogen supplies, limits the use of available nitrogen by ruminal microorganisms (Huntington, 1997).

Although the inclusion of lipid in diets can be one source of energy, it decreases ruminal organic matter (OM) digestibility and changes the

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site of digestion to the lower parts of the gastrointestinal tract (Plascencia et al., 2003). Consequently, short-chain fatty acid (FA) production in the rumen can be reduced, thereby reducing the risk of metabolic disorders and improving ruminal fermentation parameters (Aschenbach et al., 2011). Moreover, a decrease in the population of protozoa, which predate on ruminal bacteria, may occur when lipids are supplemented, thereby promoting bacterial growth (Kayouli et al., 1986).

Currently, there is a lack of information on starch supplementation combined with whole soybean (oil source) on the rumen microbial populations and fermentation parameters of Nellore steers finishing by grazing tropical grass. The hypothesis of the present study is that when combined with oil, soybean hulls could replace corn as a source of energy and improve fermentation parameters and ruminal microbiota without affecting feed intake. In this study, we accordingly evaluated the effects of starch level [corn (high) or soybean hulls (low)] in supplement with or without whole soybean (oil source) on intake, digestibility, rumen microbial population, and fermentation parameters in Nellore steers grazing *Brachiaria brizantha* cultivar Xaraés.

2. Materials and methods

The protocol used in this experiment was in accordance with the guidelines of the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal) and was approved by the Ethics, Bioethics, and Animal Welfare Committee (Comissão de Ética e Bem Estar Animal) of the São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, campus (Protocol no. 021119/11).

2.1. Animals and management

The experiment was conducted at the UNESP (Jaboticabal, SP, Brazil) from May to July 2013, during the dry season. Under the international Köppen classification, the climate is characterized as tropical type Aw with summer rains and a relatively dry winter. The local altitude is 595 m above sea level, at 21°15′22″S, 48°18′58″W. The average maximum and minimum annual temperatures are 29.1 °C and 16.5 °C, respectively, and the average annual precipitation is 105 mm, with 85% of the rainfall occurring between the months of October and March.

A replicated 4 × 4 Latin square experiment, using eight (two steers per treatment) ruminal cannulated Nellore steers (514 kg ± 30) at 24 months of age, was used to evaluate the effects of starch level in supplement, with or without whole soybean (oil source), on intake, nutrient digestibility, ruminal pH, ammonia-N (NH_3-N) and volatile fatty acid (VFA) concentrations, and ruminal microbiology over four 21-d periods. Each period consisted of 14 d for adaptation to the supplement and 7 d of sampling.

Initially, the animals were weighed, identified, treated against ecto- and endoparasites by administration of ivermectin (Ivomec, Merial, Paulínia, BR), and allocated to four paddocks, each of 0.25 ha. The pasture area was established in 2011, with planting of *B. brizantha* cultivar Xaraés. During the initial study, there was a single application of fertilizer to each paddock, as $N:P_2O_5:K_2O$ (20:05:20) at a rate of 200 kg/ha, at the end of the rainy season (May 2013). The paddocks were fitted with smooth wire fencing, waterers (with free access for the animals), and a pair of individual feed bunks.

The experimental treatments were ground corn (high starch) combined with whole soybean; corn without whole soybean; soybean hulls (low starch) combined with whole soybean; and soybean hulls without whole soybean. Crude glycerin was used in all supplements [28% of dry matter (DM)] as an energy source. Crude glycerin is a byproduct of the biodiesel agroindustry and can be used in ruminant diets without compromising intake or performance (Parsons et al., 2009; Drouillard, 2012). The crude glycerin [83.90% glycerol, 1.75% ether extract (EE), 4.30% ash, and 12.01% water] used in the present study was acquired from a soybean oil-based biodiesel production company (Cargill, Três Lagoas, Mato Grosso do Sul, Brazil). The proportions of ingredients and chemical compositions of supplements are presented in Table 1.

Table 1
Chemical composition of the ingredients, ingredient proportions and chemical composition of supplements and pasture (% DM basis).

Chemical composition	Corn	Soybean meal	Soybean hulls	Whole soybean
Dry matter	89.79	91.34	90.56	91.23
Organic matter	98.76	93.69	95.71	95.11
Crude protein	9.61	50.27	15.36	41.37
ANDFom	14.65	22.04	53.75	18.82
Ether extract	4.94	1.71	1.60	19.80
Gross energy, MJ/kg *DM*	20.42	19.71	18.16	23.64

Item[a]	High starch		Low starch		Pasture[b]
	Oil	No oil	Oil	No oil	
Ingredient proportions					
Corn	18.5	31.0	0.00	0.00	–
Soybean meal	0.00	38.5	0.00	37.0	–
Soybean hulls	0.00	0.00	18.5	32.5	–
Whole soybean	51.0	0.00	51.0	0.00	–
Crude glycerin	28.0	28.0	28.0	28.0	–
Commercial premix[c]	2.50	2.50	2.50	2.50	–
Chemical composition					
Dry matter	90.2	89.3	90.3	89.4	–
Organic matter	92.3	92.2	91.7	91.3	92.6
Crude protein	22.9	22.3	23.9	23.6	12.1
ANDFom	12.7	11.1	21.9	27.1	60.4
Starch[d]	17.2	24.7	4.45	3.29	–
Ether extract	12.4	3.62	11.8	2.58	2.16
Gross energy, MJ/kg *DM*	21.2	19.3	20.8	18.6	18.9
Metabolizable energy, MJ/kg *DM*[e]	10.5	10.0	10.5	9.62	–

[a] High starch = supplement with corn; Low starch = supplement with soybean hulls; Oil = supplement with whole soybean and No Oil = supplement without whole soybean.
[b] Average and standard deviation of the mean of samples obtained by technique of simulated grazing in five periods.
[c] 120 g Calcium, 30 g phosphorus, 25 g sulfur, 80 g sodium, 330 mg copper, 950 mg manganese, 1220 mg zinc, 24 mg iodine, 20 mg cobalt, 6 mg selenium, and 300 mg fluorine.
[d] Calculated based on ingredient values from Valadares Filho et al. (2010).
[e] Metabolizable energy = total apparent digestibility of gross energy × 0·82.

Animals were provided with experimental supplements at the rate of 1.0% body weight (BW), daily at 10:00 a.m., in a part of the feed bunks arranged in each paddock, and had *ad libitum* access to water. The BW of individual steers was recorded at the initiation of each period without a fasting period, to adjust the amount of supplement provided.

Forage mass in each paddock was estimated in each period during the grazing study. The average sward height was estimated by taking readings at 50 sampling points in each paddock, using a stick with cm graduations (Barthram, 1985). Every 21 d, forage was collected from paddocks at four sites with average sward height to represent the mean forage mass of the paddocks. At these sites, all forage included within the perimeter of a rising plate (0.25 m^2) was collected by clipping at 5 cm above soil level. The clipping samples were dried to a constant weight under forced air at 55 °C. The dry weights of these clippings were multiplied by the paddock area to estimate the forage mass. Paddocks had an average forage mass of 8350.9 kg/ha ± 865.7 and an average sward height of 32.0 cm ± 5.7. The grazing method used was the continuous grazing system (Allen et al., 2011), and the initial average sward height was 39.0 ± 4.6 cm. Forage samples were collected to be representative of the diets consumed by grazing steers from all pastures in each period during the grazing studies by hand plucking to mimic the forage selected by grazing steers (Johnson, 1978). Hand plucking was performed on the same days as the estimation of dry