Animal Feed Science and Technology 226 (2017) 39–47

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

Effect of ground soybean and starch on intake, digestibility, performance, and methane production of Nellore bulls

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ARTICLE INFO

Keywords:
Bos indicus
Carcass
Corn
Greenhouse gases
Soybean
Soybean hulls

ABSTRACT

It was hypothesized that replacement of corn with soybean hulls as the energy source, combined with ground soybean as the lipid source, would reduce methane emissions from feedlot animals without affecting their performance. This study aimed to evaluate the effects of including ground soybean combined with either a high or a low level of starch on intake, digestibility, performance, and methane emission of young Nellore bulls (n = 28, initial weight = 395 ± 32 kg) in the feedlot during the finishing phase. Diet treatments consisted of high (HS; approx. 250 g/kg) or low (LS; approx. 110 g/kg) starch levels, with (WSB) or without (NSB) soybean. Ground soybean was added as a lipid source to diets HS-WSB, LS-WSB, HS-NSB, and LS-NSB, representing 58.7, 64.6, 24.8, and 31.4 g/kg ether extract in the total dry matter, respectively. Animals were distributed in a completely randomized design, in a 2 × 2 factorial arrangement. After 119 days, the animals were slaughtered. Animals fed soybean demonstrated an average reduction of 11% in their intakes of dry matter, organic matter, and crude protein (P = 0.01). There was a significant effect of the association between starch and soybean on neutral detergent fiber intake (P = 0.01). Diets containing soybean affected the digestibility of the animals, reducing the apparent digestibility of dry matter by 3% (P = 0.02) and the digestibility of organic matter by 2.8% (P = 0.03). A significant interaction was detected between starch and soybean on the apparent digestibility of ether extract (P = 0.02) and neutral detergent fiber (P = 0.04); the lowest values for ether extract (750 g/kg) and neutral detergent fiber (432 g/kg) were obtained with HS-WSB. The animals that received WSB had their feed efficiency increased by 17% (P = 0.01). The enteric CH_4 emissions of the animals fed WSB reduced significantly (P = 0.01), with 28% decrease in g CH_4/d and g CH_4/y, 16% decrease in g CH_4 per kg of dry matter intake, and 23% decrease in CH_4 losses per percent of gross energy intake. The inclusion of approximately 250 g/kg of soybean in the diet of young Nellore bulls in the feedlot provides an increase in their feed efficiency and reduces their enteric methane emission, regardless of the level of starch in the concentrate.

Abbreviations: aNDF, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; BFT, backfat thickness; CG, carcass gain; CH_4, methane; CP, crude protein; DM, dry matter; DP ref, dressing percentage of the reference animals; EE, ether extract; GE, Gross energy; GHG, greenhouse gas; HCW, hot carcass weight; iNDF, indigestible neutral detergent fiber; IW, initial weight; LEA, loin-eye area; MM, mineral matter; NFC, Non-fiber carbohydrates; N-NH_3, ammoniacal nitrogen; NSB, without soybean; S, starch; SB, soybean; SF_6, sulfur hexafluoride; WSB, with soybean

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http://dx.doi.org/10.1016/j.anifeedsci.2017.02.004
Received 4 February 2016; Received in revised form 2 February 2017; Accepted 4 February 2017
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1. Introduction

The current increase in the production of greenhouse gases (GHGs) and their contribution to climate change is a major worldwide concern (Martin et al., 2010). Methane (CH_4) released by ruminants is the main GHG at the farm level (Veysset et al., 2010) and constitutes an energy loss for the animal ranging from 2 to 12% of its gross energy intake (Johnson and Johnson, 1995). Therefore, lowering enteric methane production should reduce such inefficiency as well as the environmental impact of ruminant production.

One of the strategies for reducing methane emissions is the inclusion of dietary lipids (Fiorentini et al., 2014). The addition of lipids to ruminant diets increases energy efficiency and reduces methanogenesis simultaneously. The addition of soybeans decreases ruminal protozoa (Fiorentini et al., 2013); protozoa being important producers of H_2 in the rumen, the decreased amount of protozoa reduces the availability of substrate for the metabolism of methanogens, thereby reducing CH_4 production (Martin et al., 2010). Of the various lipid sources available for ruminant nutrition in Brazil, soybean is valued for its wide availability, low cost, and high nutritive content (Barletta et al., 2012). Brazil is currently the world's largest soybean producer (USDA, 2014).

Another strategy that can be used in methane mitigation is the addition of readily fermentable starch grains to the diet, which promotes propionate production and may consequently reduce the level of methane produced (Moss et al., 2000). However, increasing the concentrations of starch in the diet may reduce the ruminal digestibility of neutral detergent fiber (NDF); this reduction can be partially explained by negative effects of higher starch intake on the ruminal bacterial populations (Ferraretto and Shaver, 2012). Soybean hulls provide an alternative energy source that represents a highly digestible fibrous ingredient (Van Soest, 1994) with minimal effects on fiber degradation (Firkins, 1997; Bach et al., 1999).

Few studies (Neto et al., 2015) to date have investigated the combined effect of starch and lipids on animal performance and methane emission. We hypothesized that replacing corn with soybean hulls as an energy source, combined with ground soybean as a lipid source, may reduce methane emissions without affecting the performance or carcass characteristics of feedlot beef cattle. The aim of this study was, thus, to evaluate the effect of including soybean combined with either a high or a low level of starch in the finishing of feedlot young Nellore bulls on their intake and digestibility, weight gain, carcass characteristics, and methane production.

2. Materials and methods

The procedures adopted in this experiment were approved by the Ethics, Bioethics, and Animal Welfare Committee (Comissão de Ética e Bem Estar Animal) of the Faculty of Agriculture and Veterinary Sciences – São Paulo State University (UNESP) protocol no. 021119/11.

The experiment was conducted in the facilities of the experimental feedlot at the Digestibility Unit of the Department of Animal Science at the Faculty of Agriculture and Veterinary Sciences at São Paulo State University, in Jaboticabal, SP, Brazil.

2.1. Experimental animals

Thirty-six young Nellore bulls with an average initial body weight of 395 ± 32 kg and an average initial age of 20 ± 3 months were used in the experiment. Of these, 8 were slaughtered to be used as reference for estimation of initial carcass weight gain, daily carcass gain, and final carcass gain relative to average daily weight gain. The remaining 28 animals were weighed, marked for identity, treated against ecto- and *endo*-parasites, and then acclimatized to the experimental diets (adopting the same treatments used on pasture) and feedlot facilities for 21 days.

Animals were kept in individual 21 m^2 (7 × 3 m) stalls containing a wooden trough and a drinker, and were allocated to experimental treatments in a completely randomized design under a 2 × 2 factorial arrangement (high or low starch; with or without ground soybean), totaling 4 treatments with 7 replicates per treatment. Total weight gain and average daily gain were determined at the beginning and end of the feedlot period, after a solid-feed deprivation period of 12 h. Animals were weighed at 30-day intervals to monitor variations in performance. After 140 days of feeding (adaptation + experimental period), the animals, with an average body weight of 590 ± 34 kg, were slaughtered in a commercial beef plant.

2.2. Diets

Diets were supplied once daily at 08:00 h. The diets were formulated to be isonitrogenous, and to provide a dry matter intake of 22.0 g/kg of body weight and an average gain of 1.30 kg/day as per Valadares Filho et al. (2010). Feed supply was adjusted daily based on the previous day's intake, maintaining orts at approximately 100 g/kg of the total provided, characterizing *ad libitum* intake.

Diet treatments consisted of high (HS; approx. 250 g/kg) or low (LS; approx. 110 g/kg) starch levels, with (WSB; approx. 62 g/kg ether extract) or without (NSB; approx. 28 g/kg ether extract) ground soybean. In HS, the main ingredient was corn, whereas soybean hulls were the main ingredient in LS; ground soybean was added as a lipid source. The roughage used was corn silage, and the roughage:concentrate ratio of the experimental diets was 400:600 g/kg (Table 1). All diets contained 100 g crude glycerin per kg of dry matter, replacing the corn or soybean hulls. Glycerin is a by-product from the biodiesel industry that can be used in ruminant diets without compromising intake or performance (Parsons et al., 2009; Drouillard, 2012).

Table 1
Proportion of ingredients and chemical composition of the experimental diets and corn silage (g/kg on a DM basis).

	High Starch		Low Starch		Corn Silage
	WSB[a]	NSB[b]	WSB[a]	NSB[b]	
Ingredient proportions					
Corn Silage	400	400	400	400	–
Corn	240	303.4	0.00	0.00	–
Soybean meal	0.00	176	0.00	157	–
Soybean hulls	0.00	0.00	206.8	238.6	–
Ground soybean	239	0.00	273	0.00	–
Crude glycerin	100	100	100	100	–
Commercial premix[c]	20.5	20.5	20.5	20.5	–
Chemical composition					
Dry matter	601	603	589	586	269
Organic matter	957	959	947	947	–
Starch[d]	240	259	121	97.8	179
Crude protein	140	147	156	152	84.5
Ether extract	58.7	24.8	64.6	31.4	24.7
Non-fiber carbohydrates	485	512	386	403	–
Total carbohydrates	758	781	726	763	–
aNDF	177	172	244	265	42.8
Lignin	84.1	78.4	87.5	83.3	–
Gross energy, MJ/kg DM	17.4	16.7	17.5	16.5	–

[a] WSB = With ground soybean.
[b] NSB = No ground soybean.
[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).

2.3. Sampling and characterization of diet

Samples of concentrates, roughage, and orts were collected at 30-day intervals and stored in a freezer. Subsequently, composite samples were made and dried in a forced-air oven at 55 °C for 72 h and then ground in a Wiley mill to pass through a 1-mm sieve. Dry matter (method 934.01; AOAC, 1990), mineral matter (MM; method 942.05; AOAC, 1990), ether extract (EE; method 954.02; AOAC, 1990), and lignin (method 973.18; AOAC, 1990) contents were quantified. Neutral detergent fiber (aNDF) was determined using α-amylase and without the addition of sodium sulfite by following the procedure of Van Soest et al. (1991), and adapted for the Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY).

Nitrogen concentration was determined in each sample by rapid combustion (850 °C), conversion of all N-combustion products to N_2, and subsequent measurement using a thermoconductivity cell (Leco® model FP-528; LECO Corporation, Michigan, USA). Crude protein (CP) was calculated by multiplying the percentage of N in the sample by 6.25. Gross energy (GE) content was determined using an adiabatic bomb calorimeter (SRPA Instrument Company 6300, Moline, IL, USA). The concentration of starch in concentrates and silage was determined according to Knudsen et al. (1987).

Samples of the corn silage were collected during each experimental trial for estimation of pH, ammoniacal nitrogen ($N\text{-}NH_3$), and chemical analyses. An aqueous extract was prepared (Kung et al., 1984) for pH and $N\text{-}NH_3$ determination; non-fiber carbohydrates (NFC) and total carbohydrates from the diets and orts were calculated according to the methodology of Cornell University, described by Sniffen et al. (1992).

2.4. Digestibility trial

Digestibility trials were performed in the middle third of each experimental period (days 20 and 21); the upper layer of feces was collected on two consecutive days from the floor of the stalls immediately after defecation by each animal. On the first day, the collection was performed in the morning, and on the second day, in the afternoon. After being identified, feces were pre-dried in a forced-air oven at 55 °C for approximately 72 h and ground through a 1-mm sieve mill. A composite sample based on the air-dry weight was made from the three samples of ground feces.

The fecal output of dry matter was estimated by the internal marker technique (Cochran et al., 1986), using the indigestible neutral detergent fiber (iNDF) marker. iNDF was obtained by the *in situ* method for 240 h (Casali et al., 2008) with subsequent extraction of the neutral detergent fiber according to Van Soest et al. (1991).

2.5. Quantification of enteric methane

Methane (CH_4) emissions were measured by the sulfur hexafluoride (SF_6) tracer gas technique (Johnson and Johnson, 1994), for which each animal was sampled daily for five consecutive days at the end of the final feeding period, so as not to compromise intake

and performance. All animals were fitted with gas-collection halters 10 days before CH_4 sampling to allow the animals to adapt, and to facilitate sampling. Brass permeation tubes filled with SF_6 with known release rates (2.23 ± 0.5 mg SF_6/d, mean ± SD) were administered orally to all the animals 72 h before CH_4 sampling to allow the marker gas to equilibrate in the rumen. Gas release rates from permeation tubes were determined prior to their placement into the rumen. The permeation tubes were maintained in a water bath at 39 °C and weighed in the laboratory for 7 weeks.

Animals were fitted with gas-collection halters connected to pre-evacuated polyvinyl chloride canisters designed to fill halfway over 24 h. Sampling began prior to feeding at 06:00 h daily, whereby each animal was removed from the paddock individually and conducted to the stockyard to facilitate sampling.

Collection canisters were positioned above the neck of each animal to reduce the risk of equipment damage and were connected to the halter by airline flexible-coil tubing. Canister pressure was measured after 24 h of collection and the halter replaced if the final pressure was beyond the expected range (from −12,000 [initial pressure] to −6000 [final pressure] PSI); higher pressure indicated probable blockage of the halter, whereas lower pressure indicated a probable leak in the system. In both situations, a new halter was placed on the animal to achieve an average absorption rate within the stipulated range; filling halfway over 24 h.

Pressure readings were recorded and canisters were pressurized using pure N_2. Averages of two ambient air samples in the feedlot were collected daily to determine the background concentrations of CH_4 and SF_6. The net output in exhaled air was calculated by correction against background values. The CH_4 emission rate was calculated as follows (Westberg et al., 1998): $QCH_4 = QSF_6 \times ([CH_4]_y - [CH_4]_B)/[SF_6]$, in which QCH_4 is the methane emission rate in g/d; QSF_6 is the known release rate of SF_6 from the permeation tube; $[CH_4]_y$ and $[SF_6]$ are the concentrations of respective gases in the canister, and $[CH_4]_B$ is the background concentration of methane in ambient air. From these data, CH_4 emissions were calculated per day, per year, and as a function of average daily gain, carcass gain, dry matter intake, aNDF intake, and gross energy intake.

2.6. Slaughter and evaluation of carcass

Slaughter was performed in a commercial abattoir under federal inspection, according to the animal welfare norms. The carcass of each animal was divided into two half-carcasses and weighed to determine the hot carcass weight (HCW). Carcasses were then chilled in a cold room at 0 °C for 24 h. After weighing, the left half-carcass was sectioned between the 12th and 13th ribs, and the *longissimus* muscle was used for measurements of backfat thickness (BFT) and loin-eye area (LEA). A digital caliper was used to determine BFT, and a proper square grid was used for LEA, with measurements in square centimeters (cm^2).

Hot carcass yield was calculated as the ratio between hot carcass weight and body weight of each fasted animal in percentage. The daily carcass gain (CG) was obtained based on the reference animals:

$$CG = [HCW - (IW \times DP\ ref)]/days;$$

where HCW = hot carcass weight of experimental animals, IW = initial weight of experimental animals, and DP ref = dressing percentage of the reference animals.

2.7. Statistical analyses

The experimental design was completely randomized in a 2 × 2 factorial arrangement (2 levels of each factor), and data were analyzed using the MIXED procedure of SAS software (SAS Inst. Inc., Cary, NC). The model included the fixed effects starch and lipid source with interaction:

$$Y_{ijk} = \mu + S_i + O_k + [S_i \times O_k] + e_{ijk};$$

where Y_{ijk} = observation of animal j subjected to starch i at soybean inclusion k, μ = overall mean, S_i = effect of starch i = 1 and 2, O_k = effect of soybean inclusion k = 1 and 2, $S_i \times O_k$ = interaction between starch i and soybean inclusion k, and e_{ijk} = residual experimental error.

Homogeneity of the data was verified using the UNIVARIATE procedure of SAS. Studentized residuals were plotted against the predicted values using the plot procedure to analyze data for outliers. Results were subjected to analysis of variance with means compared by Tukey's test at 5% level of significance.

3. Results

There was no significant effect of the interaction between the factors (soybean and starch) on the variables shown in Table 2. Animals fed WSB demonstrated an 11% average decrease in intake of dry matter, organic matter, crude protein, and total digestible nutrients ($P = 0.01$). Inclusion of HS in the diet increased intake rates of dry matter ($P = 0.04$), organic matter ($P = 0.02$), and total digestible nutrients ($P = 0.03$) by 7% and GE intake ($P = 0.02$) by 10%, as compared to LS diets (Table 2).

A significant effect of interaction ($P = 0.01$) between starch and soybean on aNDF intake was detected, with the lowest value in the animals fed HS-WSB (Table 3). The WSB diets affected the digestibility of animals, reducing the apparent digestibility of dry matter by 3% and organic matter by 2.8%, as compared to that in animals fed NSB diets. The apparent digestibility of protein was not affected by any diet ($P > 0.05$).

Inclusion of HS or LS did not affect ($P > 0.05$) the apparent digestibility of nutrients in any treatment (Table 2). A significant

Table 2
Effect of diets containing high and low starch, with or without soybean (soy ground or no soy ground) on intake and digestibility of Nellore bulls finished in feedlot.

	Soybean		Starch			*P*-value		
	With	No	High	Low	SEM	SB[1]	S[2]	SB × S
Intake, g/kg of BW								
Dry matter	16.4[b]	18.4[a]	18.2[a]	16.6[b]	0.12	0.01	0.01	0.69
Intake, kg/d								
Dry matter	8.31[b]	9.55[a]	9.26[a]	8.60[b]	0.82	0.01	0.04	0.49
Organic matter	7.92[b]	9.07[a]	8.84[a]	8.14[b]	0.78	0.01	0.02	0.46
Crude protein	1.23[b]	1.45[a]	1.35	1.32	0.11	0.01	0.47	0.66
Ether extract	0.52[a]	0.27[b]	0.38	0.41	0.04	0.01	0.10	0.10
Gross energy, MJ/d	145	153	157[a]	141[b]	6.54	0.23	0.02	0.75
Digestibility, g/kg DM								
Dry matter	689[b]	712[a]	702	700	2.38	0.02	0.74	0.44
Organic matter	704[b]	725[a]	713	714	2.40	0.03	0.93	0.51
Crude protein	634	641	629	647	2.66	0.51	0.06	0.09

[1]SB = Soybean; [2]S = Starch.
[a–b]Homogeneous subsets within rows as determined by Tukey HSD at the 5% level.

interaction was apparent between starch and soybean for the apparent digestibility of ether extract ($P = 0.02$) and aNDF ($P = 0.04$; Table 3). The feed efficiency of animals consuming WSB increased ($P = 0.01$) by 17%; however, no effect of any dietary treatment was observed on average daily gain, carcass yield, carcass gain, loin-eye area, and backfat thickness ($P > 0.05$; Table 4).

The enteric CH_4 emissions of animals fed WSB reduced significantly, with a 28% decrease in daily and yearly CH_4 emissions ($P = 0.01$), 27% decrease in g CH_4 per kg of average daily gain ($P = 0.01$), 22% decrease in g CH_4 per kg of carcass gain ($P = 0.01$), 16% decrease in g CH_4 per kg of dry matter intake ($P = 0.01$), 14% decrease in g CH_4 per kg of aNDF intake ($P = 0.03$), and 23% decrease in CH_4 losses per percent of gross energy intake ($P = 0.01$). Dietary starch content (HS or LS) did not affect ($P > 0.05$) enteric CH_4 production (Table 5).

4. Discussion

Addition of ground soybean to the diets of young Nellore bulls was associated with decreases in the intake and digestibility of nutrients and CH_4 emissions during the present study, regardless of the level of dietary starch. The addition of ground soybean as a lipid source was shown to have a significant effect on the intake of DM, OM, CP, and aNDF. This is consistent with previous experiments in which a decrease in DM intake was observed in feedlot cattle fed diets containing soybean (Bassi et al., 2012; Fiorentini et al., 2014).

Animals fed WSB, during the present study, had a higher EE intake (62 g/kg DM) than those fed NSB diets (28 g/kg DM). Diets containing 70 g or more of EE/kg DM may cause depression of feeding and may be toxic to rumen microorganisms, because the excess lipid adheres to food particles, creating a physical barrier that impedes the activity of microorganisms and microbial enzymes, especially when a large proportion of unsaturated fatty acids is contained in the EE (Palmquist and Jenkins, 1980; Sullivan et al., 2004). Higher EE intake of WSB fed animals might have also been caused by increased serum concentrations of unsaturated fatty

Table 3
Effect of diets containing high and low starch, with or without soybean (soy ground or no soy ground) on digestibility of EE, aNDF and intake of aNDF of Nellore bulls finished in feedlot.

		Starch			*P*-value		
		High	Low	SEM	SB[1]	S[2]	SB × S
Intake, kg/d							
aNDF	With SB	2.32[A,b]	2.63[B,a]	0.25	0.01	0.01	0.01
	No SB	2.52[A,b]	3.32[A,a]				
Digestibility, g/kg DM							
Ether extract	With SB	751[A,b]	876[A,a]	3.85	0.07	0.01	0.02
	No SB	758[A,b]	812[B,a]				
aNDF	With SB	431[B,b]	538[A,a]	3.12	0.01	0.01	0.04
	No SB	496[A,b]	553[A,a]				

[1]SB = Soybean; [2]S = Starch.
[a–b]Homogeneous subsets within rows as determined by Tukey HSD at the 5% level.
[A–B]Homogeneous subsets within columns as determined by Tukey HSD at the 5% level.

Table 4
Effect of diets containing high and low starch, with or without soybean (soy ground or no soy groun) on initial and final body weight, average daily gain (ADG), feed efficiency, carcass yield, carcass gain, loin-eye area and backfat thickness of Nellore bulls finished in feedlot.

	Soybean		Starch			*P*-value		
	With	No	High	Low	SEM	SB[1]	S[2]	SB × S
Initial body weight, kg	425	432	426	431	37.1	0.63	0.70	0.93
Final body weight, kg	588	592	593	587	55.3	0.88	0.75	0.99
Average daily gain, kg/d	1.37	1.39	1.41	1.36	0.18	0.81	0.46	0.44
Feed efficiency[3]	0.17[a]	0.14[b]	0.15	0.16	0.01	0.01	0.56	0.70
Carcass yield[4]	58.3	58.8	58.8	58.3	1.52	0.38	0.41	0.65
Carcass gain, kg/d	1.00	1.08	1.08	1.00	0.11	0.07	0.07	0.29
Loin-eye area, cm^2	84.9	86.3	89.5	81.7	11.6	0.74	0.08	0.27
Loin-eye area[5]	25.0	24.8	25.5	24.2	2.61	0.82	0.20	0.22
Backfat thickness, mm	6.70	6.22	6.04	6.88	1.85	0.50	0.24	0.51

[1]SB = Soybean; [2]S = Starch; [3]kg ADG/kg DM intake; [4]kg/100 kg of carcass; [5]cm^2/100 kg of carcass.
[a–bb]Homogeneous subsets within rows as determined by Tukey HSD at the 5% level.

acids, which activate the receptors in the satiety center of the hypothalamus (Allen, 2000; Obici et al., 2002). Therefore, it can be inferred that the differences in the intake of DM and nutrients observed during the present study were a result of the differential lipid intake of the animals.

The association between starch (high and low) and a lipid source may not influence DM intake, and an alteration in this component depends on the level of lipid inclusion (Neto et al., 2015). This reinforces the idea that the effect of lipid addition on intake is highly variable, depending mainly on the source and amount utilized, the category of animals under study, and the form of supply, among other characteristics.

The significant decrease in aNDF intake observed in animals fed HS-WSB diets during the present study was due to the lower aNDF content of this diet compared with the LS diet. Differences in the aNDF content of diets were due to the high percentage of aNDF in the soybean hulls. Dietary soybean addition reduced the digestibility of DM (3%) and OM (2.8%) in animals fed on both HS and LS WSB diets. This was probably due to a decrease in the number of rumen protozoa and bacteria, including several populations of fibrolytic bacteria, and decreased activity of fiber-degrading enzymes (Huws et al., 2010; Patra, 2013). Fibrolytic bacteria are among those most sensitive to inhibition by dietary lipid (Nagaraja et al., 1997).

The higher aNDF content of LS diets, with or without soybean addition, increased the apparent digestibility of EE and aNDF during the present study. In addition to the fact that LS diets contained lower non-fiber carbohydrate contents, this may have been due to a lower degradability of aNDF from the soybean hulls serving to increase the retention time of the digesta in the reticulo-rumen and decrease the rate of passage through the gastrointestinal tract (Oliveira et al., 2007). Also the presence the polyunsaturated fatty acids have been shown to have a toxic effect on some rumen bacteria species, especially cellulolytic bacteria (Maia et al., 2007; Mao et al., 2010). Therefore, the lower rumen digestion of aNDF in WSB in comparison to NSB can be explained by a decline in cellulolytic bacteria number notwithstanding the decrease in their fibrolytic activity.

The effects of lack of dietary starch and soybean on the average daily gain, carcass yield, CG, LEA, and BFT during this study may have been related to the nutritive value of the soybean hulls, which was similar to that of the corn. Possible alterations in rumen fermentation, associated with the different carbohydrate sources, did not affect the efficiency of use of the feeds during growth of the different animal tissues. Effects of soybean hulls on animal performance may be related to the inclusion rate of this feed ingredient. Performance is not compromised at low inclusion rates, as the hulls are more digestible than corn. Thus, when included at low percentage in the concentrate, soybean hulls can reduce metabolic disorders, thereby increasing the availability of energy from other

Table 5
Effect of diets containing high and low starch, with or without soybean (soy ground or no soy ground) on enteric CH_4 emission of Nellore bulls finished in feedlot.

	Soybean		Starch			*P*-value		
	With	No	High	Low	SEM	SB[1]	S[2]	SB × S
g/d	119[b]	164[a]	141	142	19.3	0.01	0.84	0.93
kg/yr	43.4	60.0	51.4	51.9	14.2	0.01	0.84	0.93
g/kg ADG[4]	87.5[b]	120[a]	102	105	15.9	0.01	0.54	0.43
g/kg CG[5]	121[b]	153[a]	131	142	18.9	0.01	0.13	0.42
g/kg DMI[6]	14.5[b]	17.3[a]	15.3	16.6	2.69	0.01	0.21	0.62
g/kg OMD[7]	21.4[b]	25.1[a]	22.7	24.2	4.86	0.02	0.19	0.58
g/kg aNDFI[8]	48.9[b]	57.0[a]	58.6[a]	47.3[b]	9.61	0.03	0.10	0.11
% of GEI[9]	3.49[b]	4.52[a]	3.76	4.25	0.77	0.01	0.10	0.79

[1]SB = Soybean; [2]S = Starch; [4]ADG = average daily gain; [5]CG = carcass gain; [6]DMI = dry matter intake; [7]OMD = organic matter digestible; [8]aNDFI = NDF intake; [9]GEI = gross energy intake.
[a–bb]Homogeneous subsets within rows as determined by Tukey HSD at the 5% level.

dietary components (Neto et al., 2015).

Enteric CH_4 emission expressed as g CH_4/d was reduced by 28% in animals fed upon WSB diets, independent of the starch level. It represented an energy profit, i.e., these animals consumed less and gained the same weight as others. It was probably due to retention of energy that was not wasted as CH_4, and was reflected in the increase in feed efficiency (0.17). The first explanation of the CH_4-mitigating effect of fatty acids is that, regardless of their nature, fatty acids decrease the amount of organic matter fermented in the rumen if they replace a proportion of dietary carbohydrates (Martin et al., 2016). In fact, this was evident in our study because the diets with soybean decreased dry matter intake and consequently, decreased the amount of organic matter fermentable in the rumen. CH_4 is produced in ruminants as a by-product of OM fermentation in the rumen and hindgut; this CH_4 is an energy loss to the animal and will vary with feed composition and intake (Alstrup et al., 2015).

Lipid supplementation reduces the fermentable substrate and can decrease organic matter and fiber degradability (Knapp et al., 2014). As a consequence, the amount of hydrogen produced during fermentation is reduced, resulting in decreased CH_4 production. Besides, supplementation with lipid may reduce the activity of ruminal methanogens and protozoal numbers (Hristov et al., 2013). An increased degree of fatty acid unsaturation will increase the negative effect on fibrolytic bacteria and methanogens (Giger-Reverdin et al., 2003). Free fatty acids, with their surfactant properties, can disrupt membrane integrity, impair nutrient uptake, and inhibit membrane enzyme activity and energy production, leading to cell death (Desbois and Smith, 2010). Gram-positive bacteria have been reported to be more susceptible to free fatty acids than Gram-negative bacteria (Desbois and Smith, 2010). In addition to the outer cell membrane and cell wall, other cell envelope structures, such as the glycocalyx, may affect the susceptibility of a microbe to free fatty acids. The dietary environment might also affect the toxicity of a free fatty acid to different groups of microbes. However, according to Morgavi et al. (2010), the number of methanogens is not the key factor affecting CH_4 emission. A modulation of the activity and diversity of methanogens might be responsible for the CH_4-mitigating effect of fatty acids.

Another explanation for decreased CH_4 emission is that protozoa act as physical hosts for methanogens and during fermentation produce a high quantity of H_2, which is used by the methanogens to reduce CO_2 to CH_4 (Morgavi et al., 2010). The toxic effect of its fatty acids on protozoa, associated with lower H_2 availability to methanogens, appears to be involved in the mitigation of CH_4 in ruminants by soybean. Addition of soybean or soybean oil to cattle diets has been shown to reduce rumen protozoa populations by up to 80% (Fiorentini et al., 2013). Removal of protozoa (defaunation) from the rumen is often associated with increased supply of microbial protein and improved animal productivity (Patra and Saxena, 2009). Therefore, manipulating the H_2 in the rumen is key to controlling CH_4 emission, and consequently, the metabolic pathways involved in the formation and utilization of H_2, and the methanogenic population; these are important considerations for the development of strategies to control CH_4 emission by ruminants.

In the present study, the average enteric CH_4 emission across all treatments was 51.6 kg CH_4/y and, specifically for WSB treatments as the lipid source, 43.4 kg CH_4/y. These values were lower than the 61 kg CH_4/y estimated by the IPCC (2006) for male cattle in Latin America. Though studies have demonstrated that dietary composition affects ruminant GHG production, the IPCC, which is responsible for the development of methodologies to estimate global emission stocks, only distinguishes between diets with more than 900 g/kg concentrate and diets with less than 900 g/kg concentrate. Energy lost as CH_4 emissions (expressed as a percentage of energy intake) was 3.49 and 4.52% in animals fed WSB and NSB, respectively. These values are lower than those reported by the Intergovernmental Panel on Climate Change (IPCC, 2006) for animals consuming diets with less than 900 g/kg concentrate (6.5% of the GE intake). According to Martin et al. (2007), CH_4 losses appear to be relatively constant for diets containing 300–400 g/kg concentrate (6–7% of GE intake), rapidly declining for diets containing 800–900 g/kg concentrate (2–3% of GE intake).

Brazilian livestock has been criticized for emitting significant amounts of GHGs. Possibly, the difference between these values is due to the fact that the methodologies used to estimate the emission of GHGs do not fully correspond to the national reality. In some cases, the methodology used by IPCC (2006) overestimate the emission of GHGs in cattle. It occurs because the numbers of IPCC (2006) are absolute, and it does not take into account the characteristics of each country. The IPCC (2006) itself suggests that regional studies should be performed on this issue. Such criticism has been based on the low zootechnical indices reported in animal husbandry systems, based on degraded pastures or that are below their production potential. An inefficiency of this exploration model has generated higher GHG results per kilogram of meat and/or milk produced.

Tools are being tested (e.g., lipid use, pasture management) to manipulate the rumen and to create management techniques that can reduce CH_4 emission, and, consequently, lower energy losses. For example, Carvalho et al. (2016, Neto et al. (2015 and Fiorentini et al. (2014 found lower values of energy losses when compared to those cited by IPCC (2006). Therefore, we realized that the better the quality of the diet, lower is the CH_4 emission in comparison to the IPCC default. It should be borne in mind that these tools must be linked to the economic sustainability of the producer. In the future, producers who get committed to environmental sustainability and use mitigation strategies may be rewarded with carbon credits.

Research shows that intensifying the production systems can reduce the enteric emission of CH_4, even though the emission of N_2O can be increased by the use of nitrogen fertilizers, either for grain cultivation or for pasture fertilization. This reduction of GHG per unit of product is mainly related to better utilization of the food and reduction of the age at slaughter. The overall estimation of gas balance is quite complex and requires a lot of research, including more efficient production systems such as silvopastoral and agrosilvopastoral systems that seek a better use of the gases emitted by the activities.

Knowledge of the mechanisms of CH_4 synthesis and the factors affecting CH_4 production is important. A major challenge for the ruminant production industry is to develop diets and management strategies that minimize relative CH_4 production, providing greater production efficiency, and a reduction in the contribution of livestock to global warming. The results of the present study, whereby a roughage:concentrate ratio of 400:600 g/kg was employed, and an average 4% GE loss as CH_4 emission was observed for animals fed diets including soybean, corn, or soybean hulls with corn silage, may be important for future IPCC estimates of global

emission inventories. Thus, diet modification appears to be a promising method by which the cattle industry can reduce its contribution to GHG emissions.

5. Conclusion

The inclusion of approximately 250 g of soybean/kg DM in the diet of feedlot young Nellore bulls results in an increase in feed efficiency and reduces enteric CH_4 emission, regardless of the level of starch in the concentrate, without affecting carcass characteristics.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

The authors thank the São Paulo Research Foundation (FAPESP, grants #2011/00060-8; #2013/02418-2; #2014/09033-1; #2016/08585-6) for providing financial support.

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