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Performance and methane emissions of Nellore steers grazing tropical pasture supplemented with lipid sources

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ABSTRACT - The objective of this study was to evaluate the effect of lipid sources on voluntary intake, digestibility, performance, and CH_4 emission of Nellore steers grazing *Brachiaria brizantha* cv. Xaraés forage in the dry season. Forty-five Nellore steers with average weight of 442±34 kg were alloted into one of the five treatments: without additional fat; with palm oil; with linseed oil; with protected fat; and with whole soybeans. The supplements were provided daily and quantities were adjusted to 1% of body weight and diets were formulated in accordance with the Cornell Net Carbohydrate and Protein System. The experimental design was completely randomized with five treatments and two replications. There were no effects on dry matter, organic matter, and neutral detergent fiber intake with the inclusion of lipids in the diet. The neutral detergent fiber showed decreased digestibility in animals receiving linseed oil and palm oil treatments compared with animals receiving the diet without additional fat. The linseed oil treatment reduced CH_4 emissions by 38% when expressed in mg/d/kg BW and tended to reduce the emission in g/d/kg $BW^{0.75}$. Lipid sources did not affect the weight gain of the animals. The intake and performance of grazing Nellore steers supplemented at 1% body weight with lipid sources were not modified. However, fiber digestibility was reduced with palm or linseed oil addition. Linseed oil reduced enteric CH_4 emissions. Linseed oil has the potential to reduce enteric CH_4 emissions in continuous tropical grazing systems based on *B. brizantha* grass.

Key Words: linseed oil, palm oil, protected fat, supplementation, whole soybeans

Introduction

The production of beef cattle in tropical countries is generally associated with grazing cultivated pastures and low production costs. However, increased competitions in feed prices for better meat quality as well as the growing concern about emissions of greenhouse gases are causing changes in the beef production system in Brazil and in the world.

Methane (CH_4) and CO_2 are natural byproducts of microbial fermentation of carbohydrates and, to a lesser extent, aminoacids in the rumen and the hindgut of farm animals (Hristov et al., 2013). Methane emissions represent a loss of about 5 to 7% of dietary gross energy (to as low as 3% in cattle fed high-grain diets) and are about 16 to 26 g/kg of dietary dry matter intake (DMI) (could be lower with diets containing very high proportions of grain) (Hristov et al., 2013). Various strategies can be used to reduce enteric CH_4 production (Hristov et al., 2013), highlighting the inclusion of dietary lipids (Martin et al., 2010). The addition of lipids to ruminant diets has also been recommended, as it similarly increases energy efficiency and hence reduces methanogenesis. Although greater concentrations of fats substantially decrease methane production, they often exert detrimental effects on fiber digestion and, consequently, on animal performance (Patra, 2013).

Supplementation of unsaturated fatty acids (UFA) exerts deleterious effects on methanogens and protozoa and reduces the acetate:propionate ratio in the rumen (Macmüller et al., 1998). In this manner, there is a reduction in production of ruminal enteric CH_4 and the intensity in which this inhibition occurs is determined by the degree of fat saturation and the supplemented amount (Fievez et al., 2003). These disturbances are attributed mainly to modifications in the rumen microbial ecosystem (Doreau and Chilliard, 1997). Although polyunsaturated

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fatty acids (PUFA) may decrease protozoa and promote cellulolysis (Doreau and Ferlay, 1995), the prevailing action is the modification of microbial membrane permeability, causing metabolic disorders, mainly in cellulolytic flora (Maia et al., 2007).

Many products with different fatty acid profiles can be used as fat sources in diets for ruminants (Fiorentini et al., 2014; Neto et al., 2015). However, there are few studies that assess the effect of lipids upon CH_4 emission mitigation and upon performance of zebu cattle grazing tropical pasture. Furtheremore, diets rich in fiber could result in increased rates of lipolysis and hydrogenation (Jenkins, 1993) and cause less toxicity to microorganisms due to reduction in the level of dietary PUFA (Broudiscou et al., 1994).

We hypothesized that the inclusion of lipids in supplements for grazing cattle reduces CH_4 emissions without affecting performance and that more unsaturated sources would cause greater reductions. In this manner, this work aimed to evaluate intake, digestibility, performance, and emission of enteric CH_4 in Nellore steers supplemented with lipid sources and grazing *Brachiaria brizantha* cv. Xaraés forage during dry season.

Material and Methods

The study was carried out in Jaboticabal, SP, Brazil, located at 21°15'22" S latitude, 48º18'58" W longitude and 595 m altitude. According to the Köppen classification, the climate of Jaboticabal is tropical, Awa type, with rainy summers and dry winters. The protocol used on this experiment was in accordance with the COBEA (Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the CEBEA (Comissão de Ética e Bem Estar Animal) of the FCAV-UNESP - Jaboticabal campus (case no. 012799).

Forty-five castrated Nellore steers with average initial body weight of 442±34 kg and eight months of age were used. The animals were kept in 1.9-ha paddocks of *Brachiaria brizantha* cv. Xaraés pasture under continuous grazing system with a variable stocking rate (put and take), maintaining a sward height of 35 cm. The animals were distributed in a completely randomized design (five animals per paddock and two paddocks per treatment) with two replicates per treatment.

The formulations of diets were specified using RLM/Esalq-USP software (Lanna et al., 1999) and were formulated for maximum optimization of animal performance. The treatments consisted of supplemental lipid sources in concentrate: without additional fat; with palm oil derived from the palmaceae plant *Orbignya oleifera,* which has a lipid profile rich in medium-chain fatty acids (lauristic and myristic); with linseed oil; with protected fat (Lactoplus - Dalquim group, Itajaí, Santa Catarina, Brazil); and with whole soybeans. The supplements were provided daily at 1% of body weight in an open trough at 08.00 h. Supplements were formulated (Table 1) to reach 10% of ether extract in dry matter of supplement, except for treatment without additional fat, which was 4.1%.

To determine the weight gain, animals were weighed at the beginning and at the end of the experiment and every 28 d to adjust the amount of supplement provided. Steers were fasted (feed and water) for 14 h, for initial and final weight measurements.

The estimation of individual feed intake was performed by the two-marker method: LIPE® and indigestible acid detergent fiber (iADF), used to estimate the excretion of fecal matter (as dry weight) and forage intake, respectively. Fecal dry matter excretion was determined using purified and enriched lignin (LIPE®) described by Santos et al. (2011). The animals received the marker daily for six days of the digestion trial. After three days of adaptation, fecal samples were collected daily directly from the rectum, at 16.00, 11.00, and 07.00 h on the first, second, and third day of collection, respectively. Samples of feces from each animal on each day of collection were individually used to determine the concentrations of LIPE®.

The forage intake was estimated based on fecal production data using the iADF of the forage obtained from

Table 1 - Supplement and pasture compositions (g/kg on a DM basis)

	Supplement				
	WF	PO	LO	PF	WS
Ingredient					
Ground corn	755	655	655	645	570
Soybean meal	200	220	220	220	0.00
Palm oil	0.00	80.0	0	0.00	0.00
Linseed oil	0.00	0.00	80.0	0.00	0.00
Protected fat[1]	0.00	0.00	0.00	90.0	0.00
Whole soybeans	0.00	0.00	0.00	0.00	400
Urea	15.0	15.0	10.0	10.0	0.00
Minerals[2]	30.0	30.0	30.0	30.0	30.0
Chemical composition					
Dry matter, g/kg	880	890	890	890	890
Organic matter, g/kg DM	942	942	943	920	937
Crude protein, g/kg DM	220	219	214	224	224
Ether extract, g/kg DM	41.0	104	94.0	97.0	116
Neutral detergent fiber, g/kg DM	126	119	149	118	129
Lignin, g/kg DM	11.0	23.0	23.0	28.0	30.0

WF - without additional fat; PO - palm oil; LO - linseed oil; PF - protect fat; WS - whole soybeans; DM - dry matter.
[1] Lactoplus® (Dalquim group, Itajaí, Santa Catarina, Brazil).
[2] Mineral supplement Bellman, Bellboi (Ca, 160 g; P, 40 g; Mg, 5 g; S, 40 g; Na, 160 g; Cu, 945 mg; Mn, 730 mg; Zn, 3,500 mg; I, 70 mg; Co, 56 mg; Se, 18 mg; F (max), 400 mg.

the simulated grazing on the 41st day of the trial period. The quantification of fecal iADF on forage and supplement samples was obtained after a 264-h ruminal incubation of samples as described by Casali et al. (2008). Acid detergent fiber analysis was performed with the ANKOM200/220 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA). The supplement intake consisted of the total supplement provided divided by the number of animals per paddock, since there were no observed supplement leftovers in the troughs.

Pasture samples were collected during each period with the hand-plucking technique. Samples of the pasture, concentrates, and feces were oven-dried at 55 °C for 72 h and ground in a Wiley mill to pass through a 1-mm screen. Procedures described by the Association of Official Analytical Chemists (AOAC, 1990) were used to determine dry matter (DM; method 934.01) and mineral matter (MM; method 942.05) and to obtain an acid ether extract (EE; method 954.02). Nitrogen was determined using an LECO FP-528 nitrogen analyzer (LECO Corp., St. Joseph, MI, USA).

Content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were based on the procedures described by Mertens (2002) using the ANKOM200/220 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA). The NDF analyses were performed without sodium sulphite, with alpha amylase and corrected for residual ash. The NDF and ADF were also corrected for residual ash. The acid detergent lignin was determined by solubilization of the cellulose with sulfuric acid according to Van Soest et al. (1991).

The gross energy content of feeds (pasture and concentrate) was determined using an adiabatic bomb calorimeter (PARR Instrument Company 6300, Moline, IL, USA). The acid detergent lignin was determined by solubilization of the cellulose with sulfuric acid according to Van Soest et al. (1991).

To determine the fatty acid composition of feed offerings, a sample of approximately 1 g was used. The frozen sample was homogenized in 20 mL of a chloroform and methanol solution (2:1) using a Turrax homogenizer, disintegrator, and emulsifier (Folch et al., 1957). In the next step, the lipid-extracted aliquot was methylated using the protocol of Kramer et al. (1997). Fatty acids were quantified (Table 2) using a GC 2010 gas chromatograph (Shimadzu Corp., Kyoto, Japan) with an SP-2560 capillary column (100 m × 0.20 mm i.d. with a 0.02-µm film thickness) (Supelco, Bellefonte, PA). The initial temperature was set to 70 °C for 4 min (13 °C/min) until it reached 175 °C and then held for 27 min. After this, the temperature was increased 4 °C/min until it reached 215 °C and was held there for 31 min. Hydrogen was used as the carrier gas with a flow of 40 cm^3/s.

The apparent digestibility coefficients (DC) of DM, OM, and NDF were determined according to the values obtained for intake, fecal output, and diet and fecal composition, following the equation: DC = (NI – NF) / (NI × 100), in which NI = nutrient intake (kg) and NF = nutrient in the feces (kg).

To evaluate CH_4 emission, 20 animals were chosen (four animals per treatment). The selection criterion was the tameness of the animal and easeness to be handled in the management center (stockyard). Each animal received a pair of SF_6 capsules with average emissions of 2.78±1.18 mg/d and 610 mg of load for the whole set. The air expelled by the animals was sampled for five consecutive days, for 24 h per d, from the 78th d of the experimental period.

The basal concentrations of SF_6 and CH_4 were determined daily from ambient air samples collected by two air samplers in the experimental paddocks. The direct measurement of ruminal CH_4 technique was used, in which SF_6 was used as tracer gas according to the methods described by Johnson et al. (1994). The flow of CH_4 produced by the animals was calculated in relation to the tracer gas flow (SF_6) from the permeation rate of the inserted capsule in the rumen, accounting for the baseline concentrations of CH_4 and SF_6 present in the air:

$$Q_{CH4} = Q_{SF6} * ([CH_4]_Y - [CH_4]_B)/[SF_6],$$

in which Q_{CH4} = rate of methane emission by the animal; Q_{SF6} = known rate of SF_6 emission; $[CH_4]_Y$ = methane concentration in the air sampler; $[CH_4]_B$ = methane

Table 2 - Fatty acid profile of supplements and pasture

	Supplement					Pasture
	WF	PO	LO	PF	WS	
Fatty acid, g/100 g						
C6:0 (caproic)	0.00	0.11	0.00	2.70	0.00	0.00
C8:0 (caprlic)	0.00	0.00	0.00	0.00	0.00	0.00
C10:0 (capric)	0.00	2.43	0.00	0.00	0.00	0.36
C12:0 (lauric)	0.00	37.6	0.10	4.91	0.00	1.86
C14:0 (miristic)	0.14	12.6	0.04	0.04	0.02	1.31
C15:0 (pentadecanoic)	0.00	0.00	0.00	0.00	5.87	0.00
C16:0 (palmitic)	10.4	8.98	7.18	2.79	2.27	30.6
C18:0 (stearic)	3.68	2.21	4.41	14.8	6.21	3.63
C18:1 (oleic)	21.8	17.1	20.5	44.8	23.5	4.33
C18:2 (linoleic)	48.9	14.9	23.0	25.8	54.4	15.1
C18:3 (linolenic)	4.76	1.27	41.9	1.27	5.34	36.9
Others	10.4	2.80	2.80	2.78	2.31	5.80
Saturated fatty acids	46.3	72.6	20.4	33.9	21.4	42.7
Unsaturated fatty acids	53.6	27.4	79.6	66.1	78.5	57.3

WF - without additional fat; PO - palm oil; LO - linseed oil; PF - protected fat (Lactoplus® Dalquim group, Itajaí, Santa Catarina, Brazil); WS - whole soybeans.

concentration in the blank; and [SF_6] = sulfur hexafluoride concentration in the air sampler.

The CH_4 and SF_6 concentrations were determined by gas chromatography in the laboratories in a HP6890 gas chromatograph equipped with flame ionization detector (FID) at 280 ºC, Plot HP-Al/M megabore column (0.53 µm, 30 m; for CH_4), an electron capture detector at 300 °C, and HP-MolSiv megabore column (0.53 mm × 30 m × 25.0; for SF_6), with two loops of 0.5 cm^3 attached to two six-way valves. The gas chromatograph oven was kept at 50 °C during the analysis and heated at 150 °C for approximately 15 min to clean the column.

Data of intake, digestibility, CH_4 measurement, and performance were analyzed in a completely randomized design using the MIXED procedure in SAS (Statistical Analysis System, version 9.0). The least square means were compared using the Tukey test. Differences between treatments were considered significant at P<0.05 and trends were discussed at P<0.10.

For intake, digestibility, and performance, initial body weight was used as a covariate. The statistical model used was:

$$Yij = \mu + Ti + \beta 1 + \varepsilon ij \; \varepsilon ij \sim iidN(0,\sigma 2),$$

in which Yij = intake, digestibility, and weight gain from treatment i and paddock j; µ = general mean; Ti = effect of treatment i (i = 1, 2, 3, 4, 5); β1 = covariate for initial body weight (IW); and εij = experimental error (j = 1, 2).

For enteric CH_4 emission, the following statistical model was used:

$$Yij = \mu + Ti + \varepsilon ij \; \varepsilon ij \sim iidN(0,\sigma 2),$$

in which Yij = enteric CH_4 emission from treatment i and paddock j; µ = general mean; Ti = effect of treatment i (i = 1, 2, 3, 4, 5); and εij = experimental error (j = 1, 2).

For the repeated measures of forage traits, analysis of variance was adopted, using the MIXED procedure and the repeated option in SAS. The model included effects of the treatment, month of evaluation, and the interaction month × treatment, as described in:

$$Yijk = \mu + Ti + Pj + (TP)ij + \varepsilon ijk \; \varepsilon ijk \sim iidN(0,\sigma 2),$$

in which $Yijk$ = forage traits from treatment i on period j and paddock k; μ = general mean; Ti = effect of treatment i (i = 1, 2, 3, 4, 5); Pj = effect of period j (j = 1, 2, 3, 4); $(TP)ij$ = effect of the interaction between treatment i and period j; and εijk = experimental error (k = 1, 2). Different covariance structures of the residuals were tested to determine the structure that best adjusted for each trait. The matrices for each variable were chosen according to the BIC (Bayesian Information Criteria), in which the smallest value of BIC is used as selection criterion.

Results

The canopy height was kept between 35 and 25 cm, with an average stocking rate of 2.4 AU/ha. During the experimental period, the pasture DM content increased over time (P<0.001) while the protein content decreased (P<0.001). The NDF (P = 0.663) and EE (P = 0.659) contents remained unchanged, whereas the ADF content was higher (P<0.001) only in August (Table 3). The acid detergent insoluble protein expressed relative to the amount of CP was higher (P<0.001) in the month of August compared with the other periods.

The total DM intake (DMI) (P = 0.397), forage DM (FDM) (P = 0.406), OM (P = 0.398), CP (P = 0.174), and NDF (P = 0.081) were not affected by the inclusion of lipids in the diet. Animals fed palm oil had higher (P = 0.001) intake of saturated fatty acids (SFA). The intake of UFA was higher (P = 0.001) in the treatment with whole soybeans, followed by treatment with linseed oil and with protected fat (Table 4). Animals supplemented with linseed oil had reduced apparent digestibility of DM (P = 0.024) and OM (P = 0.027) compared with protected fat and whole soybean treatments. The NDF digestibility was negatively affected (P = 0.002) by palm oil and linseed oil (Table 4).

No treatment effects were observed for average daily weight gain (ADG) (P = 0.797) and enteric CH_4 emissions, expressed in kilograms of CH_4 per year (kg/year) (P = 0.217), grams of CH_4 per day (g/d) (P = 0.217), grams of CH_4 per kilogram of DM intake (g/kg DMI) (P = 0.161), and energy loss as CH_4 (% gross energy intake) (P = 0.157). There was, however, a reduction in CH_4 emissions in milligrams of CH_4 per day per kilogram of body weight (mg/d/kg BW) (P = 0.044) and a reduction trend of grams of CH_4 per day

Table 3 - Chemical composition of available pasture in different months of the year

	Month				SEM	P-value
	May[1]	June[2]	July[3]	August[4]		
Composition						
DM, g/kg	327d	426c	500b	615a	12.6	<0.001
OM, g/kg of DM	910a	908a	897b	897b	11.3	<0.001
CP, g/kg of DM	142a	131b	114c	86.6d	1.90	<0.001
NDF, g/kg of DM	523	517	507	515	8.71	0.663
ADF, g/kg of DM	294b	293b	299b	329a	3.44	<0.001
Lignin, g/kg of DM	50.2b	50.7b	68.4a	84.4a	3.09	<0.001
EE, g/kg of DM	32.0	36.7	37.3	29.3	3.25	0.659
ADIP, g/kg of CP	118b	119b	140b	174a	6.53	<0.001

[1] May 24th.
[2] June 11th and 22nd.
[3] July 9th and 27th.
[4] August 19th.
DM - dry matter; OM - organic matter; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; EE - ether extract; ADIP - acid detergent insoluble protein; SEM - standard error of the mean.
Means followed by different letters are significantly different at P<0.05 by Tukey's test.

per kilogram of metabolic weight (g/d/$BW^{0.75}$) (P = 0.087), in which the linseed oil supplement reduced CH_4 emissions by 38% compared with the control treatment (Table 5).

Discussion

Reductions in DMI with inclusion of lipids in the diet are often observed (Bateman II and Jenkins, 1998; Wanapat et al., 2011). This effect is usually due to the interference of fatty acids in fiber digestibility (Jenkins, 1993) and inhibition of appetite due to the increase in serum concentrations of unsaturated fatty acids that activate satiety center receptors in the hypothalamus (Allen, 2000). However, in this experiment, the addition of lipid sources in supplements did not affect the DM and nutrient (OM, CP, and NDF) intakes. These results confirm the observations by Allen (2000), who compiled experiments using lipid sources (oilseeds, unprocessed animal fat, hydrogenated fatty acids and triglycerides, and calcium salts of palm oil) in diets of lactating cows. The author found inconsistent effects on DM intake, in which lipids reduced intake on only a few occasions.

Table 4 - Composition of intake and digestibility of lipid-supplemented feeds consumed by Nellore steers

	Supplement					SEM	P-value
	WF	PO	LO	PF	WS		
Intake, kg/d							
Total dry matter	11.5	11.6	11.0	12.0	12.8	0.57	0.394
Forage dry matter	6.70	6.98	6.09	7.14	7.93	0.59	0.406
Organic matter	10.5	10.7	10.1	10.9	11.8	0.52	0.398
Crude protein	1.79	1.81	1.76	2.02	1.97	0.07	0.174
Neutral detergent fiber	4.59	4.54	4.38	4.74	5.38	0.17	0.081
Fatty acid intake, g/d							
C6:0 (caproic)	0.01b	0.81b	0.01b	18.8a	0.01b	0.69	<0.001
C10:0 (capric)	0.07b	18.1a	0.03b	0.06b	0.06b	0.80	<0.001
C12:0 (lauric)	0.12b	279a	0.64b	34.2b	0.11b	12.5	<0.001
C14:0 (myristic)	0.38b	93.2a	0.24b	0.32b	0.16b	4.14	<0.001
C15:0 (pentadecanoic)	0.13b	0.11b	0.08b	0.11b	52.9a	1.06	<0.001
C16:0 (palmitic)	26.5b	67.3a	39.2b	20.1b	21.0b	4.44	0.003
C18:0 (stearic)	9.31c	16.5c	23.9c	103a	55.9b	4.27	<0.001
C18:1 n9,c (oleic)	54.9c	127c	112c	313a	212b	15.4	<0.001
C18:2 n6,c (linoleic)	122b	111b	125b	180b	489a	18.7	<0.001
C18:3 n6 (linolenic)	11.9b	9.48b	227a	8.86b	48.0b	10.1	<0.001
Saturated fatty acids	204bc	485a	190c	283b	251bc	13.2	0.001
Unsaturated fatty acids	193c	230c	438b	408b	539a	13.4	0.001
Total intake	398b	716a	629a	691a	790a	27.9	0.003
Digestibility, kg/kg							
Dry matter	0.59ab	0.59ab	0.55b	60.0a	0.61a	0.008	0.024
Organic matter	0.64ab	0.64ab	0.60b	0.66a	0.66a	0.007	0.027
Neutral detergent fiber	0.59a	0.46b	0.48b	0.62a	0.62a	0.01	0.002

WF - without additional fat; PO - palm oil; LO - linseed oil; PF - protected fat (Lactoplus® Dalquim group, Itajaí, Santa Catarina, Brazil); WS - whole soybeans.
SEM - standard error of the mean.
Means followed by different letters are significantly different at P<0.05 by Tukey's test.

Table 5 - Initial and final weights, average daily weight gain, and methane emissions of Nellore steers on feed supplemented with lipids

	Supplement					SEM	P-value
	WF	PO	LO	PF	WS		
Initial weight, kg	440	430	443	450	444	17.4	0.734
Final weight, kg	494	481	501	502	497	19.9	0.797
ADG, kg/d	0.59	0.57	0.65	0.58	0.59	0.05	0.797
CH_4, kg/year	41.5	41.1	25.6	37.2	30.1	4.82	0.217
CH_4, g/d	114	112	70.2	101	82.4	13.2	0.217
CH_4, mg/d/kg BW	238a	228a	147c	208ab	181b	16.7	0.044
CH_4, g/d/kg ($BW^{0.75}$)	1.12	1.08	0.69	0.98	0.84	0.09	0.087
CH_4, kg/kg gain	0.24	0.24	0.14	0.23	0.18	0.03	0.304
CH_4, g/kg DMI	9.51	9.91	7.26	8.74	6.61	0.88	0.161
CH_4,% GEI	3.39	3.36	2.48	2.89	2.27	0.31	0.157

WF - without additional fat; PO - palm oil; LO - linseed oil; PF - protect fat (Lactoplus® Dalquim group, Itajaí, Santa Catarina, Brazil); WS - whole soybeans; SEM - standard error of the mean; ADG - average daily gain; BW - body weight; $BW^{0.75}$ - metabolic weight; DMI - dry matter intake; GEI - gross energy intake.
Means followed by different letters are significantly different at P<0.05 by Tukey test.

The inclusion of fat in the diet of ruminants can interfere with the digestibility of nutrients, especially fiber, as observed in this study for treatments with palm oil and with linseed oil. Fiber digestibility reduction has also been observed in diets with linseed oil by Broudiscou et al. (1994), Martin et al. (2008), and Eugène et al. (2011). The PUFA are more toxic to rumen microorganisms due to the number of double bonds in the structure of fatty acids (Jenkins, 1993). The absence of effects on fiber digestion with addition of other sources rich in UFA (soybean fat and protected fat) can be attributed to the low reactivity of protected fat in the rumen and the rigid outer layer in soybeans (Coppock and Wilks, 1991). The seed coat of grains can hinder the bacterial lipolytic action on triglycerides resulting in reduced release of UFA and lesser effect on ruminal microbiota.

It is expected, therefore, that sources with more SFA profile (palm oil) are less harmful to fiber digestibility and intake (Wanapat et al., 2011). In contrast, this was not observed, since the palm oil treatment reduced the fiber digestibility in a manner similar to a source rich in UFA (linseed oil). The medium-chain fatty acids such as lauric and myristic acids found in high amounts in the palm oil diets may be toxic to the bacteria and methanogenic archaea in the rumen (Soliva et al. 2004a and 2004b); and the dissociation of these acids in bacterial cells has been proposed as a mode of antimicrobial action (Goel et al., 2009). Manso et al. (2006) also observed a negative effect on NDF digestibility with increasing level of palm oil included in diets of sheep.

Lipid inclusion in ruminant diets increases the energy density and can result in better performance (Nelson et al., 2004; Rosa et al., 2013). However, fatty acids can reduce ruminal fermentability and potentially affect the performance of the animal. Thus, it is possible that the reduction in nutrient usage in the rumen caused by addition of lipids may have been compensated by the greater energy density of the diet, resulting in no detectable effect upon performance. Gillis et al. (2004) and Luden et al., (2009) also found no effect of fat addition on beef cattle performance.

The estimated enteric CH_4 emission was of 41 kg/year in the control diet. This value was lower than that reported by IPCC (2006), which estimated an average production of 56 kg/year in cattle. The CH_4 emission was of approximately 10 g per kilogram of DMI in the diet without additional fat, a lower value than that observed in most studies with beef cattle using the SF_6 tracer technique. Neto et al. (2015) showed emission values of 15.8 g/kg DMI in Nellore bull in pasture fed supplements containing high or low starch with or without soybean grain. These values are far higher than those found in this study. Barbero et al. (2015) found emission values between 10.8 to 19.5 g/kg DMI in accordance with treatment (short height (15-cm grass grazing), high supplementation (0.6% of BW), or tall height (35-cm grass grazing), without supplementation).

Energy loss as CH_4 expressed in percentage of energy consumed was of 2.9% on average. Cattle CH_4 emission represents energy loss of around 6% in grazing animals and 3.5% in confined animals (IPCC, 2006). According to the presented results, we can infer that the inclusion of concentrate supplement in grazing animals can reduce energy losses, resulting in an efficiency of energy utilization similar to those observed in confined animals (Fiorentini et al., 2014), corroborating Neto et al. (2015). In the evaluation of energy loss in cattle fed only grass, Kurihara et al. (1999) found a 10.9% energy loss and Pelve et al. (2012) observed that CH_4 emissions from non-lactating cows and heifers fed forage harvested from semi-natural heterogeneous forages correspond to an average of 8.9% of gross energy intake.

The linseed oil treatment decreased emission of CH_4 by 38% compared with the diet without supplemental source of lipid (mg/d/kg BW). This reduction was similar to the 33% observed by Chung et al. (2011) and higher than the 27% observed by Martin et al. (2008) and the 18% observed by Beauchemin et al. (2009), using linseed as a lipid source and corn silage as forage. All diets with lipid sources contained, on average, 34 g of additional lipid per kilogram of DM and the reduction of CH_4 emissions in animals supplemented with linseed oil was of 4 g per kilogram of DM intake. There was therefore a reduction of 1.15 g of CH_4 per kg of DM intake for every 10 g of supplemented lipid in the diet (linseed oil). This value corroborates the results found by Grainger and Beauchemin (2011) on a meta-analysis evaluation of the effect of different lipid sources on CH_4 emission, in which they concluded that, for cattle, an increase of 10 g fat per kg of DM in diet reduces CH_4 emission in 1 g per kilogram of DMI. The percentage reduction was of 12.6% for every 1% of lipid added. The other evaluated sources showed no reduction in the enteric CH_4 emissions compared with the control diet.

Grazing animals have the potential to emit more CH_4, due to the higher fiber content and acetate:propionate ratio in the rumen. Diets rich in fiber increase the lipolysis and hydrogenation rates (Jenkins, 1993), while maintain lower concentrations of UFA and, thus, decrease toxicity to microorganisms caused by the added fat (Broudiscou, 1994; Bateman and Jenkins, 1998). The inhibitory response of fats on methane production depends on concentration, type, fatty acid composition of fats, and nutrient composition of diets (Beauchemin et al., 2008). Greater concentrations of fats do substantially decrease methane production, but often

exert detrimental effects on digestibility and fermentation of feeds including animal performance (Patra, 2013). Therefore, Chung et al. (2011) investigated the potential effects of feeding ground linseed on enteric CH_4 production and showed that including ground linseed in a barley silage-based diet can mitigate enteric CH_4 emissions, but not in a grass hay-based diet.

The effectiveness of linseed in reducing ruminal methanogenesis in this experiment with grazing animals is consistent with that reported in the literature on confined animals (Eugène et al., 2008; Beauchemin, et al., 2009; Martin et al., 2010), with the results reported in this work. According to Morgavi et al. (2010), the mitigation of rumen methanogenesis can be achieved by reducing the H_2 supply to the methanogenics obtained from favoring the production of propionate and the methanogenic archaea and protozoa (H_2 producers). Thus, the direct action of linseed oil on microbial population was probably responsible for reduction in CH_4 emission compared with animals that received no source of supplemental lipid.

Supplements rich in PUFA, such as linoleic and linolenic acids, have a mitigating effect on CH_4 emission in the rumen (Martin et al., 2010). Maia et al. (2007) attributed this effect to the rupture of bacteria membrane integrity caused by unsaturation of the molecules of these fatty acids. Animals supplemented with linseed oil consumed, on average, 352 g of linoleic and linolenic acids every day. This represents more than double the intake of these acids in supplements without additional fat, palm, and protected fat (average of 80 g/d). Only the supplementation with soybean allowed intake of C18:2 and C18:3 greater than with linseed (185 g/d). Although CH_4 emission was higher in animals receiving linseed oil, animals supplemented with soybeans emitted 27% less CH_4 than animals that did not receive additional lipid source in the supplement.

Conclusions

The linseed oil has the potential to reduce enteric CH_4 emissions in continuous tropical grazing systems based on *B. brizantha* grass.

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