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Ruminal fermentation and degradation, kinetic flow of the digesta and milk fatty acid composition of cows fed chopped elephantgrass supplemented with soybean oil

Fermentação e degradação ruminal, cinética de fluxo da digesta e perfil de ácidos graxos do leite de vacas alimentadas com capim-elefante picado suplementado com óleo de soja

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Abstract

The aim of this study was to evaluate the ruminal parameters of fermentation and degradation, kinetic flow of rumen digesta, and milk fatty acid composition of cows fed 52% chopped elephantgrass-based diets containing 0.0% (control), 1.5%, 3.0% and 4.5% soybean oil (SO) on a dry matter (DM) basis. Four rumen-cannulated Holstein x Gyr dairy cows with an average milk production of 15.6 ± 3.0 kg day^{-1} and 90 ± 25 days in milk were allocated in a 4 x 4 Latin square design. The results were analyzed by mixed models. Significant differences were declared at $P \leq 0.05$, and P-values from $0.05 < P \leq 0.10$ were considered as a trend. The inclusion of SO in the diet had no effect on the ruminal pH or total volatile fatty acid concentration, but there was a quadratic effect on the ruminal ammonia nitrogen content and a trend for a linear reduction (P=0.07) in the molar proportion of rumen acetate. Linear reductions were also observed in the DM and neutral detergent fiber (NDF) effective degradabilities of elephantgrass forage, but the fluid and particulate passage rates in the rumen and the DM and NDF intakes were unchanged by SO inclusion in the diet. Milk production, protein and lactose contents and yields were unaltered by dietary SO levels. There were linear reductions in the milk fat and total solids contents, but there was no effect of dietary treatments on their yields. The inclusion of soybean oil in the diet of Holstein x Gyr cows fed chopped elephantgrass improved the nutritional quality of milk fat as a result of increased contents of oleic, rumenic and vaccenic acids, which are beneficial to human health, and a concomitant reduction in hypercholesterolemic saturated fatty acids such as lauric, myristic and palmitic acids.

Key words: Conjugated linoleic acid. *Pennisetum purpureum*. Rate of passage. Ruminal metabolite.

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Resumo

O objetivo deste estudo foi avaliar os parâmetros de fermentação e degradação ruminal, a cinética de fluxo da digesta, e o perfil de ácidos graxos (AG) do leite de vacas alimentadas com 52% de capim-elefante picado e inclusão de 0,0 (controle), 1,5, 3,0 e 4,5% de óleo de soja (OS) na matéria seca (MS) da dieta. Foram utilizadas quatro vacas Holandês x Gir com 90 ± 25 dias em lactação, produzindo 15,6 ± 3,0 kg dia^{-1} de leite, canuladas no rúmen, e alocadas em delineamento Quadrado Latino 4 x 4. Os resultados foram analisados por modelos mistos. Efeitos foram considerados significativos quando P <0,05, enquanto valores de P entre 0,05 e 0,10 foram considerados indicativos de tendência. A inclusão de OS na dieta não alterou o pH do rúmen, nem a concentração total de ácidos graxos voláteis, mas promoveu efeito quadrático sobre a concentração de N amoniacal, e tendência de redução linear (P=0,07) sobre a proporção molar de acetato. Foram observadas reduções lineares nas degradabilidades efetivas da MS e da fibra em detergente neutro (FDN) do capim-elefante, mas não houve alteração nas taxas de passagem ruminal das fases líquida e sólida, nem nos consumos de MS e FDN. A produção de leite, os teores e as produções de proteína e lactose não foram alteradas pela inclusão de OS na dieta, mas houve redução linear nos teores de gordura e de sólidos totais do leite, mas sem efeito sobre as suas produções diárias. A inclusão de óleo de soja em dietas baseadas em capim-elefante picado melhorou a qualidade nutricional da gordura do leite, com aumento nos teores dos ácidos oleico, vacênico e rumênico, benéficos à saúde, e redução dos teores dos ácidos graxos hipercolesterolêmicos láurico, mirístico e palmítico.

Palavras-chave: Ácido linoleico conjugado. Metabólito ruminal. *Pennisetum purpureum*. Taxa de passagem.

Introduction

The milk fat of ruminants contains biologically active fatty acids (FAs), whose consumption promotes human health. Among them, the rumenic acid *cis*-9, *trans*-11 CLA, the main isomer of CLA (conjugated linoleic acid) in ruminant milk, is highlighted. Anticarcinogenic, antidiabetogenic (type 2 diabetes), antiatherogenic and immunomodulatory properties have been attributed to this isomer (YANG et al., 2015). As the main source of rumenic acid in the human diet is ruminant milk fat (YANG et al., 2015), the production of milk enriched with this FA and other health-enhancing FAs, such as vaccenic (*trans*-11 C18:1), oleic (*cis*-9 C18:1) and α-linolenic (*cis*-9, *cis*-12, *cis*-15 C18:1), has been the subject of research carried out in Brazil (LOPES et al., 2015) and worldwide (KLIEM; SHINGFIELD, 2016).

Well-managed tropical grasses, used for grazing or cutting, contain high levels of linoleic (*cis*-9, *cis*-12 C18:2) and α-linolenic polyunsaturated-FAs (LOPES et al., 2015), which are the main substrates for the formation of vaccenic acid in the rumen. In the mammary gland, vaccenic acid is the precursor for the synthesis of 70% to 95% of all rumenic acid secreted in bovine milk (KLIEM; SHINGFELD, 2016). In addition, the production of milk enriched with rumenic, vaccenic and oleic bioactive FAs and concomitantly with lower contents of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) saturated FAs, which are considered hypercholesterolemic (FAO, 2010), may be potentiated by lipid supplementation of the diet of the cow with free vegetable oils, such as soybean and sunflower, which are rich in linoleic acid (LOPES et al., 2015).

Even in diets formulated with an ether extract (EE) content below the recommended maximum limit for lactating cows (< 7% of diet DM) (NRC, 2001), high dietary levels of vegetable oils rich in free polyunsaturated-FAs may cause changes in parameters of ruminal fermentation (RODRIGUES et al., 2007) and promote a reduction in digestibility and/or intake of DM and the neutral detergent

fiber (NDF) (BATEMAN; JENKINS, 1998; RODRIGUES et al., 2017), with a negative effect on the milk fat content and milk yield (RODRIGUES et al., 2017). In addition, it may cause an increase in the formation of FAs intermediates of ruminal biohydrogenation, such as *trans*-10, *cis*-12 CLA (EIFERT et al., 2006) and *trans*-9, *cis*-11 CLA (RIBEIRO et al., 2014), which are associated with the inhibition of lipogenesis in the mammary gland of cows (BERNARD et al., 2008; SHINGFELD et al., 2010).

The present study provides results on the association between nutrient metabolism in the rumen and the milk FA profile in cows fed rations based on chopped elephantgrass (*Pennisetum purpureum*, Schum.) supplemented with soybean oil. In Brazil, such results are important given the lack of technical and scientific information about the production of milk that is naturally enriched with FAs beneficial to human health but contains low levels of atherogenic saturated FAs. The absence of this information impairs the marketing of milk or dairy products to consumers interested in products with such characteristics. In addition, elephantgrass is one of the most important tropical grasses used for cutting, and the genetic group Holstein x Gyr is one of the most prevalent involved with milk production in Brazil.

The aim of this study was to evaluate the ruminal fermentation and degradation parameters, the kinetic flow of digesta in the gastrointestinal tract, and the milk fatty acid profile in Holstein x Gyr dairy cows fed chopped elephantgrass-based diets supplemented with soybean oil.

Material and Methods

The study was carried out at Embrapa Dairy Cattle (Coronel Pacheco, MG, Brazil) from January to April 2008. Four multiparous dairy cows (458 ± 52 kg) with genetic composition varying from 3/4 to 15/16 Holstein x Gyr were used at the initial third of lactation (90 ± 25 days) and produced 15.6 ± 3.0 kg day^{-1} of milk. The cows were provided with a ruminal cannula that had an internal opening (diameter) of 110 mm (Kehl Ind. Com. Ltda., São Carlos, SP, Brazil). All of the experimental procedures with animals were carried out according to Embrapa Dairy Cattle guidelines for animal care and use in research.

A 4 x 4 Latin square (LS) experimental design was adopted. Each LS period comprised 21 days with 10 days for adaptation to the rations and 11 days for the sampling and data recording.

The experimental treatments were four isonitrogenous and isofibrous rations based on chopped elephantgrass with inclusion (DM basis) of 0.0% (control), 1.5%, 3.0% and 4.5% soybean oil (SO). The experimental rations (Table 1) were formulated according to the NRC (2001) to meet the nutritional requirements for a cow weighing 450 kg, producing 18 kg day^{-1} of milk with 3.5% fat, and gaining 0.2 kg day^{-1}.

Table 1. Ingredients and chemical composition of the experimental diets on a dry matter (DM) basis.

Item	Soybean oil levels (% of diet DM)			
	0.0	1.5	3.0	4.5
Ingredient, % of DM				
Chopped elephantgrass[a]	52.0	51.9	52.5	52.5
Corn meal	16.1	15.4	14.0	13.3
Soybean meal	16.1	15.4	14.0	13.3
Citric pulp	14.8	14.7	15.4	15.4
Soybean oil[b]	0.0	1.5	3.0	4.5
Mineral/vitamin supplement[c]	1.0	1.0	1.0	1.0
Chemical composition, % DM				
DM, % of as fed	32.7	33.1	33.7	33.1
Crude protein (CP)	12.4	12.8	12.2	12.5
Ether extract (EE)	1.6	3.0	4.2	5.3
Neutral detergent fiber (NDF)	48.5	49.1	49.4	48.9

[a]28.8% DM, 6.5% CP, 1.4% EE, 73.1% NDF, 9.8% lignin and 44.4% DM *in vitro* digestibility.
[b]Veleiro® (Cargill Agrícola S.A., Uberlândia, MG, Brazil): 53.8, 22.5 and 6.5 g 100 g^{-1} of fatty acids, respectively, of linoleic, oleic and α-linolenic acids.
[c]Top Milk Núcleo® (Matsuda, *Álvares* Machado, SP, Brazil): contained per kg: 255 g Ca, 76 g P, 20 g S, 30 g Mg, 60 mg Co, 850 mg Cu, 65 mg I, 2,000 mg Mn, 20 mg Se, 6,000 mg Zn, 1,000 mg Fe, 760 mg F, 220,000 IU of vitamin A, 500 IU of vitamin E.

The cows remained in a corral and were provided with fresh water and a mineral mixture. The corral was equipped with individual electronic troughs (American Calan, Inc., Northewood, NH, USA). The rations were provided to the cows as a total mixed ration (TMR) (roughage : concentrate ratio of 52 : 48, DM basis) once a day (08h00) immediately after the morning milking in sufficient amounts to allow for a 10% ort.

The Napier cultivar of elephantgrass was used, which was cut and chopped daily. The concentrates with SO were prepared weekly to avoid lipid peroxidation.

From the 16th to the 21st day of each LS period, the voluntary intake of each cow was measured as the difference between the daily quantity of feed that was supplied and its respective ort. Samples of the chopped elephantgrass and the ingredients of the concentrates were collected daily and stored (-10°C) for chemical analyses. Samples of the individual orts were also collected and transformed into composites by cow x LS period and stored (-10°C). At the end of the experiment, these samples were thawed, pre-dried (55°C, 72 h), milled (1 mm) and analyzed for DM, crude protein (CP), EE and NDF according to Detmann et al. (2012).

The cows were mechanically milked twice a day (05h00 and 14h00) with milk yields recorded from the 16th to the 21st day of each LS period. Aliquots of milk from each milking period (2/3 at morning milking + 1/3 at afternoon milking) were collected, and individual samples (30 mL) were stored in flasks with bronopol preservative for analysis of protein, fat, lactose and total solids. The analyses were performed by medium infrared spectrometry (Bentley 2300; Bentley Instruments, Inc., Chaska, MN, USA) at the Milk Quality Laboratory of Embrapa Dairy Cattle (Juiz de Fora, MG, Brazil). The milk urea nitrogen (MUN) content of these samples was analyzed by an enzymatic and spectrophotometric method of trans-reflectance (ChemSpeck150, Bentley Instruments, Inc., Chaska, MN, USA) at the Clínica do Leite (Piracicaba, SP, Brazil).

On the 17th day of each LS period, individual milk samples were collected and stored (-20°C) in flasks with no preservative to determine the FA profile using gas chromatography (model 6890N, Agilent Technologies, Inc., Santa Clara, CA, USA) with a 100 m x 0.25 mm x 0.2 *μm* column (CP-SIL 88; Varian, Inc., Mississauga, ON, USA) and a flame ionization detector (FID). This analysis was performed at the Chromatography Laboratory of Embrapa Dairy Cattle (Juiz de Fora, MG, Brazil). Chromatographic conditions and temperature programming were described by Cruz-Hernandez et al. (2007).

The nutritional quality of milk fat was also evaluated by the atherogenicity (AI) and thrombogenicity (TI) indexes and by the relationships between omega 6 and omega 3 FAs (ω-6/ω-3 FA ratio) and between hypo and hypercholesterolemic FAs (h/H FA ratio), according to the following equations: AI = [C12:0 + (4 * C14:0) + C16:0] / (*cis*-9 C18:1 + Σ *cis* ω-6 FA + Σ *cis* ω-3 FA); TI = (C14:0 + C16:0 + C18:0) / [(0.5 * *cis*-9 C18:1) + (0.5 * Σ *cis* ω-6 FA) + (3 * Σ *cis* ω-3 FA) + (Σ *cis* ω-3 FA / Σ *cis* ω-6 FA)]; ω-6/ω-3 FA ratio = Σ *cis* ω-6 FA / Σ *cis* ω-3 FA; and h/H FA ratio = (*cis*-9 C18:1 + Σ *cis* ω-3 FA) / (C12:0 + C14:0 + C16:0), in which Σ *cis* ω-3 FA = *cis*-6, *cis*-9, *cis*-15 α-C18:3; and Σ *cis* ω-6 FA = *cis*-9, *cis*-12 C18:2.

The total excretion of purine derivatives was calculated as the sum of the amounts of allantoin and uric acid excreted in urine and the amounts of allantoin in milk. From the 14th to the 16th day of each LS period, 10 mL urine samples obtained by spontaneous urination were acidified with 40 mL of 0.0036 N sulfuric acid and analyzed for creatinine, allantoin and uric acid concentrations. The analysis of the allantoin concentration was performed in samples of 10 mL of milk after deproteinization with 5 mL of 25% trichloroacetic acid and filter paper filtering. The calculations and laboratory analyses were performed as described by Mendonça et al. (2004).

On the last day of each LS period, after the morning milking, blood samples were collected by puncture of the coccygeal vein using vacuum tubes containing K-EDTA (Vacutainer, Becton-Dickinson, Rutherford, NJ, USA), which were immediately centrifuged (1,500 x *g*; 15 min) for plasma extraction, which in turn was transferred and stored (-20°C) in microtubes (2 mL) for glucose concentration analysis at the Chromatography Laboratory of Embrapa Dairy Cattle, performed by an enzymatic-colorimetric method in a Biochemistry Analyzer 2700 (YSI, Inc., Yellow Springs, OH, USA).

The ruminal parameters were evaluated during the first two days of each LS period. Samples of ruminal fluid were collected immediately before (time 0) and 2, 4, 6, 8, 10, 12, 16 and 24 h after the provision of the diets. They were immediately filtered using double gauze, and the pH was measured using a portable digital potentiometer. Two 10 mL aliquots were then pipetted and added to flasks with 0.4 mL of H_2SO_4 (50% v/v, subsample 1) or 2 mL of metaphosphoric acid (25%, subsample 2) and frozen. After thawing, subsample 1 was analyzed for the ammonia N concentration ($N\text{-}NH_3$) according to the INCT-CA N-007/1 method (DETMANN et al., 2012). Subsample 2 was centrifuged (17,000 x *g* for 10 min) and analyzed for the molar concentration (m*M*) of the volatile fatty acids (VFAs) acetate, propionate and butyrate using gas chromatography (model 7820A, Agilent Technologies, Inc., Santa Clara, USA) with a FID and Nukol capillary column (30 m x 22 mm x 0.25 μm) connected to free FA (SUPELCO, Bellefonte, PA, USA).

For the study of ruminal DM and NDF degradabilities (NOCEK, 1988), a pre-dried (55°C, 72 h) and milled (5-mm) sample of elephantgrass forage was used, which was conditioned in nylon bags (10 x 20 cm, 50 μ porosity, 10-20 mg of sample per cm^2) incubated in the rumen of each cow in each LS period. The nylon bags for time 0 were washed and frozen, and the remaining bags

were incubated in the rumen and removed 2, 6, 12, 24, 48 and 96 h later and frozen. Subsequently, they were washed, pre-dried (55°C, 72 h), and weighed, and the residues were analyzed for DM and NDF contents (DETMANN et al., 2012). The ruminal degradability parameters were estimated by adjusting the degradation data, as a function of time, to the nonlinear model described by Tomich and Sampaio (2004) using the NLIN procedure of SAS version 9.0. Curves were generated for each cow in each LS period. The effective degradabilities of DM and NDF were calculated (ØRSKOV; MCDONALD, 1979) based on the estimated particulate passage rates in the rumen.

In the study of the kinetics of particulate passage, the elephantgrass forage sample was subjected to hot extraction with a neutral detergent, obtaining a fibrous fraction with 83% NDF, which was complexed with sodium dichromate (UDÉN et al., 1980). For each LS period, 100 g of chromium-mordanted NDF (2% Cr, DM basis) was intra-ruminally administered in a single dose to each cow, and fecal samples were collected directly from the rectum at 6, 12, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, 84, 92, 96, 104, 120, 128 and 144 h after dosing with the marker. Fecal samples were dried in a forced-air oven at 55°C (72 h), milled to pass through a 1 mm screen and stored for analysis for Cr content using atomic absorption spectrophotometry according to the INCT-CA M-005/1 method (DETMANN et al., 2012).

For the study of the kinetics of the fluids in the rumen in each LS period, on the morning before the provision of the diets, 5 g of Co-EDTA (14% Co, DM basis) was obtained according to the procedures reported by Udén et al. (1980), diluted in 200 mL of distilled water and intra-ruminally administered to the cows. From the ruminal fluid samples collected for the study of the fermentation parameters, 5 mL was further pipetted, transferred to tubes, stored (-10°C), and then analyzed for Co content by atomic absorption spectrophotometry (UDÉN et al., 1980).

The parameters of the kinetics of particulate and fluid passage were estimated for each cow using the NLIN procedure of SAS version 9.0. The concentrations of Cr in the feces as a function of time were adjusted to the two-compartment biexponential time-independent model (GROVUM; WILLIAMS, 1973) and to the multi-compartmental model (DHANOA et al., 1985), while the Co content in the ruminal fluid was adjusted to the unicompartmental exponential model reported by Valadares Filho and Pina (2011).

The results were analyzed by mixed models using the MIXED procedure of SAS version 9.0. The ruminal fermentation parameters were analyzed as repeated measures in time and were considered to be fixed effects: treatment (level of SO in the diet), sampling time and interaction between these factors, and random effects: cow, LS period and the interaction cow*LS period*treatment. Ten matrices of covariance were evaluated, based on the Akaike information criterion. The other variables were analyzed considering treatment as a fixed effect and cow and LS period as random effects. The linear and quadratic effects of the treatments were analyzed using orthogonal contrasts. The results are reported as least squares means. Significant differences were declared at $P \leq 0.05$, and P-values from $0.05 < P \leq 0.10$ were considered as a trend. Regression equations between specific variables were adjusted by the REG procedure and Pearson correlations obtained by the procedure CORR of SAS.

Results and Discussion

The interaction treatment*sampling time was not significant ($P > 0.05$) for any of the ruminal parameters. The inclusion of SO in the diet promoted a quadratic effect ($P = 0.0116$) on the ruminal concentration of $N\text{-}NH_3$ (Table 2) with a maximum value of 15.5 mg dL^{-1} estimated for the ration with 2.3% SO. The similarities ($P > 0.05$) between diets in relation to the propionate molar ratio and ruminal concentration of total VFAs

indicate that the increase in the molar ratio of butyrate in response to inclusion of SO may be related to the reduction (P=0.0741) found for the molar ratio of acetate (Table 2). The interconversion of acetate to butyrate is an important mechanism in ruminal metabolism, since it allows for the oxidation of reduced cofactors for the continuity of the fermentation process (VALADARES FILHO; PINA, 2007). Moreover, a higher production of butyrate over acetate can help prevent ruminal pH reduction (DIJKSTRA et al., 2012), which in the present study was not altered (P>0.05) by the dietary inclusion of SO (Table 2). In diets with high levels of polyunsaturated-FAs, this is particularly important, since the main microorganisms responsible for the biohydrogenation processes of these FAs in the rumen are fibrolytic bacteria, which are sensitive to pH < 6.0 (MARTIN; JENKINS, 2002).

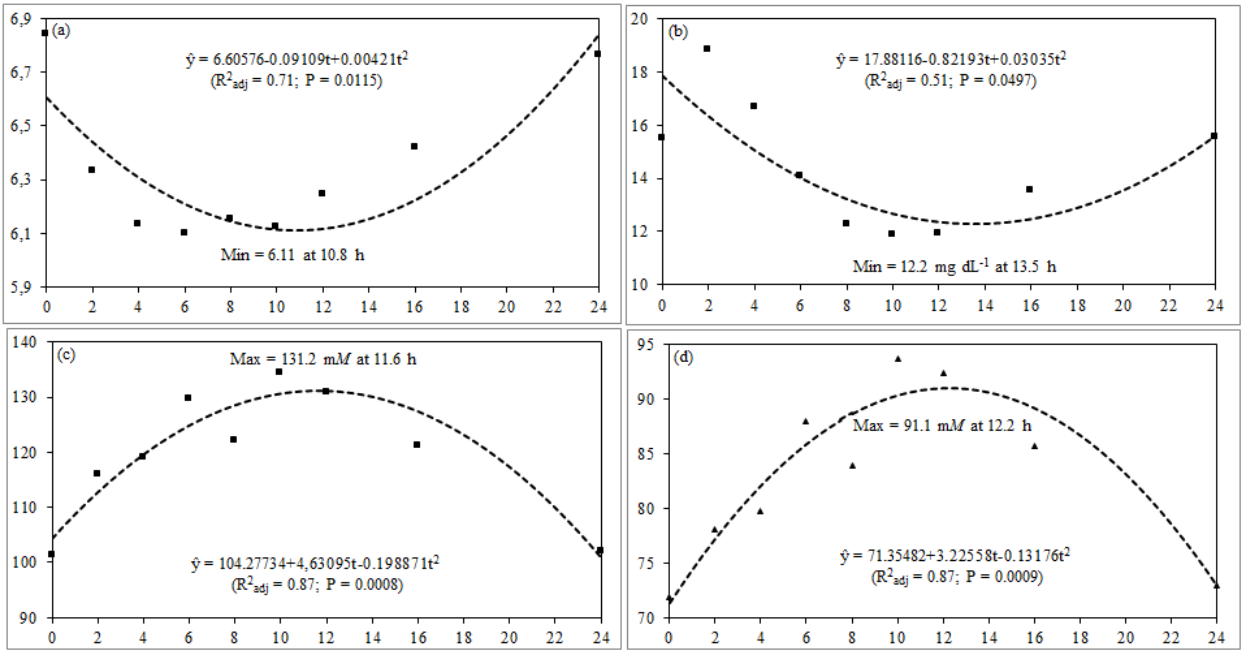
As a reflection of changes in the diet consumption patterns and fermentation of ruminal digesta during the day, a quadratic effect of the sampling time was observed on the pH (P=0.0002) and on the concentrations of $N\text{-}NH_3$, acetate, propionate, butyrate and total VFAs in the rumen (P<0.0001) (Figure 1). Thus, the gradual reduction in the ruminal concentration of $N\text{-}NH_3$ in the morning (Figure 1b), after intake of the diet by the cows, can be partially attributed to its use by the fibrolytic microbiota of the rumen, which represents the high requirement of this metabolite for fermentation of fibrous carbohydrates with concomitant production of acetate (VALADARES FILHO; PINA, 2007). This was evidenced by the similar times at which the minimum concentration of $N\text{-}NH_3$ (12.2 mg dL^{-1} at 13.5 h; Figure 1b) and maximum concentration of acetate (91.1 m*M* at 12.2 h; Figure 1d) were observed in the rumen, justifying the negative correlation between these two metabolites of ruminal fermentation (r = -0.76; P<0.05).

Table 2. Ruminal fermentation parameters in Holstein x Gyr dairy cows fed chopped elephantgrass-based diets containing increasing levels of soybean oil (SO).

Item	SO levels (% of diet DM)				Standard error of the mean	P-value	
	0.0	1.5	3.0	4.5		Linear	Quadratic
pH	6.38	6.38	6.29	6.33	0.0451	0.2514	0.6546
Ammonia N (mg dL^{-1})	13.44	15.38	15.26	13.81	1.2981	0.7194	0.0116
Total VFAs[a] (m*M*)	129.34	110.66	118.13	120.16	6.5063	0.6488	0.1332
Molar proportions (%)							
Acetate	70.51	69.81	68.89	67.89	1.2031	0.0741	0.8663
Propionate	25.21	25.89	26.46	26.74	1.1599	0.2474	0.8355
Butyrate	4.28	4.30	4.66	5.37	0.3037	0.0004	0.0576
Acetate/propionate ratio	2.86	2.73	2.64	2.58	0.1844	0.2000	0.8621

[a]Volatile fatty acids.

Figure 1. Effect of sampling time (t) on: **(a)** pH, **(b)** ammonia nitrogen, **(c)** total volatile fatty acids and **(d)** acetate contents in the rumen of Holstein x Gyr dairy cows fed chopped elephantgrass-based diets.



By contrast, there was no correlation (P>0.05) between the ruminal concentrations of N-NH_3 with those of propionate and butyrate, whose maximum values, respectively, of 33.9 m*M* and 6.4 m*M* were reached between 10.2 h and 10.6 h after feeding. These times are close to the time at which the lowest ruminal pH value was estimated (6.11 at 10.8 h; Figure 1a), justifying its high correlation with these two VFAs (r = -0.98; P<0.0001).

Lopes and Aroeira (1998) fed Holstein x Zebu cows with chopped elephantgrass supplemented with non-lipid concentrate and reported a pH of 6.33, similar to those observed in the present study (Table 2). These authors found generally lower ruminal concentrations of N-NH_3 (10.4 mg dL^{-1}), acetate (64.0 m*M*), propionate (13.6 m*M*) and butyrate (5.8 m*M*), which were justified by the lower level of production of the cows (6.1 kg day^{-1} of milk) and by the greater roughage : concentrate ratio (76 : 24) of the ration.

Although the ruminal environment of the cows could be considered adequate for the activity of the populations of bacteria that ferment fibrous carbohydrates (pH > 6.0 and N-NH_3 > 10 mg dL^{-1}; VALADARES FILHO; PINA, 2007; Figures 1a and 1b), the inclusion of SO in the diet promoted a linear reduction in the degradation rate of DM (P=0.0282) and the effective degradability (ED) of DM and NDF (P<0.05) of elephantgrass forage (Table 3); comparing the control ration with the 4.5% SO ration, the reductions in these three parameters of ruminal degradation were, respectively, 48%, 13% and 20% (Table 3).

Table 3. Parameters of ruminal degradabilities of dry matter and neutral detergent fiber in Holstein x Gyr dairy cows fed chopped elephantgrass-based diets containing increasing levels of soybean oil (SO).

Parameters[1]	SO levels (% of diet DM)				Standard error of the mean	P-value	
	0.0	1.5	3.0	4.5		Linear	Quadratic
Dry matter							
A – Potential degradability (%)	54.3	55.0	55.0	56.9	2.5017	0.1821	0.6100
c – Degradation rate (% h^{-1})	4.00	3.33	3.27	2.71	0.3772	0.0282	0.8597
ED – Effective degradability[a] (%)	33.0	30.7	31.5	29.2	2.0907	0.0096	0.9815
Neutral detergent fiber							
A (%)	48.6	48.6	47.7	52.1	2.4490	0.3334	0.3124
c (% h^{-1})	3.44	3.17	3.22	2.61	0.3548	0.1373	0.6088
ED[a] (%)	20.7	18.7	19.8	17.3	1.2946	0.0358	0.7075

[a]ED = effective degradability, calculated considering the passage rate of solids in the rumen estimated by the Dhanoa et al. (1985) model.

These results, supported by the linear reductions observed in the molar ratio of acetate (P=0.0741; Table 2) and in the purine derivatives excretion (P=0.0127; Table 4), are indicative that the inclusion of SO in the diet promoted dysbiosis in the bacterial community of the rumen (ZENED et al., 2013). There have probably been more significant reductions in the population and activity of cellulolytic strains, which are more vulnerable to the bacteriostatic effects of polyunsaturated-FAs (VLAEMINCK et al., 2006), which negatively affect the ruminal degradation of the fibrous fraction of the diet. However, in spite of the reductions observed in ED (Table 3), there was no effect on the DM and NDF intakes, which were similar (P>0.05) between treatments (Table 4). In diets with increasing levels of sunflower oil, Shingfield et al. (2008) also observed a linear decrease (P=0.07) in the degradability of the NDF, without an effect (P>0.05) on the DM intake of the cows. The DM and NDF intakes were similar to those reported by Araújo et al. (1995), respectively, of 2.9% BW and 1.6% BW, for Holstein x Zebu cows producing 8 to 12 kg day^{-1} of milk, which were fed elephantgrass silage supplemented with 6 kg day^{-1} of concentrate containing 40% ground soybeans. On the other hand, the values of ED of DM and NDF (Table 3) were lower than those reported by Lopes and Aroeira (1998), respectively, of 38.9% and 30.9%, which may be partially attributed to the better nutritional quality of elephantgrass forage (10% CP, 72% NDF) of that study. Moreover, for the calculation of ED, Lopes and Aroeira (1998) used a particulate rate of passage in the rumen of 5% h^{-1}, which is higher than those estimated in the present study (Table 5). As the diets were isonitrogenous (Table 1) and there was no effect (P>0.05) on the DM intake, the inclusion of SO did not alter the CP intake but, as expected, promoted a linear effect (P<0.0001) on EE consumption (Table 4).

Table 4. Plasma concentration of glucose, total excretion of purine derivatives (PD), daily nutrient intake and milk production and composition of Holstein x Gyr dairy cows fed chopped elephantgrass-based diets containing increasing levels of soybean oil (SO).

Item	SO levels (% of diet DM)				Standard error of the mean	P-value	
	0.0	1.5	3.0	4.5		Linear	Quadratic
Plasma glucose (mg dL^{-1})	48.3	50.5	49.4	49.4	1.4837	0.6016	0.2446
PD (mmol day^{-1})	435.0	429.3	381.2	319.6	36.1902	0.0127	0.3084
Nutrient intake (kg day^{-1})							
Dry matter	13.4	13.8	14.5	13.6	0.9527	0.4918	0.2011
Neutral detergent fiber	8.6	8.7	8.7	8.6	0.7505	0.9154	0.6759
Crude protein	1.3	1.3	1.4	1.3	0.1075	0.4645	0.1557
Ether extract	0.23	0.31	0.44	0.50	0.0265	<0.0001	0.3868
Nutrient intake (g 100 g^{-1} of body weight)							
Dry matter	2.90	3.02	3.17	2.93	0.1024	0.5668	0.1019
Neutral detergent fiber	1.86	1.90	1.90	1.85	0.0953	0.9595	0.3036
Yield							
Milk (kg day^{-1})	14.4	15.1	15.2	16.2	1.6222	0.0560	0.7579
Fat-corrected milk[a] (kg day^{-1})	13.4	13.8	13.4	14.0	1.5219	0.4328	0.8251
Fat (g day^{-1})	506.5	517.3	490.0	500.3	59.6609	0.4542	0.9851
Protein (g day^{-1})	418.3	430.0	443.0	462.3	42.3899	0.0135	0.7029
Lactose (g day^{-1})	652.5	678.8	687.0	698.8	87.9183	0.2124	0.7687
Total solids (g day^{-1})	1,695.3	1,741.0	1,744.5	1,829.5	198.76	0.1585	0.7398
Milk composition (%)							
Fat	3.50	3.45	3.22	3.07	0.1392	0.0006	0.3731
Protein	2.90	2.88	2.92	2.89	0.1109	0.9395	0.7995
Lactose	4.52	4.46	4.50	4.44	0.0975	0.0608	0.9525
Total solids	11.75	11.55	11.44	11.30	0.1397	0.0009	0.5908
Milk urea nitrogen (mg dL^{-1})	14.33	13.45	13.40	13.28	1.2495	0.3626	0.6241

[a]Fat-corrected milk (NRC, 2001) = 0.4*MilkProduction + 15*(%MilkFat/100)*MilkProduction.

Table 5. Rates of passage of fluids and solids of digesta in Holstein x Gyr dairy cows fed chopped elephantgrass-based diets containing increasing levels of soybean oil (SO).

Rate of passage (RP)	SO levels (% of diet DM)				Standard error of the mean	P-value	
	0.0	1.5	3.0	4.5		Linear	Quadratic
k_p (% h^{-1})[a]	10.13	12.41	11.09	11.76	1.4718	0.2604	0.2604
$k_{1\text{-Grovum}}$ (% h^{-1})[b]	3.99	4.43	3.89	4.39	0.3163	0.5344	0.8947
$k_{2\text{-Grovum}}$ (% h^{-1})[b]	4.97	5.44	4.80	5.34	0.3612	0.6651	0.8881
$k_{1\text{-Dhanoa}}$ (% h^{-1})[c]	2.77	2.98	2.75	2.70	0.2889	0.4744	0.3740
$k_{2\text{-Dhanoa}}$ (% h^{-1})[c]	9.19	8.80	10.06	11.37	0.7642	0.0385	0.2456

[a]k_p = RP of fluids in the rumen.

[b]$k_{1\text{-Grovum}}$ and $k_{2\text{-Grovum}}$ = respectively, RP in the rumen and RP in the caecum/proximal colon estimated by adjusting the Grovum and Williams (1973) model.

[c]$k_{1\text{-Dhanoa}}$ and $k_{2\text{-Dhanoa}}$ = respectively, RP in the rumen and RP in the caecum, estimated by adjusting the Dhanoa et al. (1985) model.

There was no effect (P>0.05) of SO inclusion in the diet on the fluid and particulate rate of passages in the rumen (Table 5). In diets based on hay of tropical grass, Bateman and Jenkins (1998) also did not observe an effect (P>0.05) of the dietary inclusion of 0, 2, 4, 6 and 8% SO (DM basis) on the fluid rate of passage in the rumen (10.30 to 10.74% h^{-1}). These authors observed lower values than those obtained in the present study (Table 5), which can be attributed to the use of non-lactating cows. In a study carried out with lactating cows that were fed corn silage-based diets, Bettero et al. (2013) also did not observe an effect of the inclusion of 2% SO in the diet on the particulate passage rate in the rumen. In general, as presented in Table 5 and corroborating the results reported by Lopes et al. (2008), the ruminal and post-ruminal particulate passage rates obtained from the adjustment of the fecal chromium excretion data to the Grovum and Williams (1973) model were, respectively, higher and lower than those estimated from the Dhanoa et al. (1985) model. According to Ellis et al. (1994), estimates of similar ruminal (k_1) and post-ruminal (k_2) passage rates ($k_1 \cong k_2$) are inconsistent with the assumptions related to two-compartment models, and based on this, they have recommended that the ratio between these two parameters ($k_2 \div k_1$) should exceed the value of 1.5 to obtain a reliable estimate of the passage rate. This recommendation allowed for Lopes et al. (2008) to conclude that the process of time-dependence implicit in the multi-compartmental model promoted a better adjustment of the fecal excretion data. These authors, who worked with lactating Holstein x Zebu cows fed chopped elephantgrass supplemented with non-lipid concentrate, reported ruminal and post-ruminal particulate passage rates, estimated from the model of Dhanoa et al. (1985), respectively, from 2.61 to 2.97% h^{-1} and from 8.17 to 10.94% h^{-1}, that is, close to those obtained in the present study (Table 5).

Fat-corrected milk production and contents and yields of protein and lactose were not influenced by the inclusion of SO in the diet (P>0.05). Lactose secretion is mainly modulated by plasma glucose synthesized via gluconeogenesis from the propionate produced in the rumen (SANTOS, 2011). Thus, the absence of an effect (P>0.05) of the inclusion of SO on rumen propionate concentrations (data not shown) and plasma glucose justify the similarity observed between treatments for the milk lactose content (Table 4).

There was also no effect (P>0.05) of the treatments on the MUN content (Table 4), whose values can be considered normal (SANTOS, 2011). On the other hand, there was a linear reduction (P=0.0006) in the milk fat content and, consequently, in the milk total solids content (P=0.0009). However, the production of these components did not change (P>0.05), since the tendency for a linear increase (P=0.056) observed in milk production in response to lipid supplementation of the diet with SO was compensated for by the reduction in milk fat and total solids contents (Table 4).

The negative effect of dietary inclusion of free vegetable oils on the milk fat content of cows is well documented in the literature (LOPES et al., 2015; RODRIGUES et al., 2017). In the present work, comparing the control ration with the 4.5% SO ration, this reduction was 14%. An exponential relationship ($\hat{y} = 35.7883^{-12.0491x} - 32.1528$; $r^2 = 0.45$; n = 16; P=0.0208) was observed between the variation in the milk fat content and the milk fat concentration of *trans*-10, *cis*-12 CLA (SHINGFIELD et al., 2010), which in turn presented a linear increase (P=0.0009) in response to the inclusion of SO in the diet (Table 6). The *trans*-10, *cis*-12 CLA is formed in the rumen from the biohydrogenation of linoleic acid and is associated with inhibition of lipogenesis in the mammary gland of cows (BERNARD et al., 2008; SHINGFIELD et al., 2010).

Table 6. Milk fatty acid (FA) composition of Holstein x Gyr dairy cows fed chopped elephantgrass-based diets containing increasing levels of soybean oil (SO).

FAs (g 100 g^{-1} FAs)	SO levels (% of diet DM)				Standard error of the mean	P-value	
	0.0	1.5	3.0	4.5		Linear	Quadratic
Linear even-chain saturated FAs							
Σ 4≤C≤10	10.03	9.50	7.51	6.37	0.4419	<0.0001	0.2611
C12:0	2.96	2.36	1.75	1.40	0.2067	<0.0001	0.1587
C14:0	11.36	9.64	7.41	6.14	0.7758	<0.0001	0.2673
C16:0	29.51	24.12	20.35	18.61	1.6641	<0.0001	0.0153
Σ 12≤C≤16	43.83	36.11	29.51	26.15	2.5297	<0.0001	0.0196
Σ 4≤C≤16	53.86	45.62	37.02	32.52	2.7330	<0.0001	0.0297
C18:0	11.08	13.15	13.78	14.21	0.7709	0.0191	0.2887
Odd linear-chain FAs							
C11:0	0.34	0.43	0.17	0.20	0.1044	0.1212	0.8541
C13:0	0.094	0.070	0.069	0.041	0.0084	0.0028	0.7971
C15:0	1.47	1.20	1.04	0.91	0.0766	<0.0001	0.0513
C17:0	0.68	0.65	0.58	0.54	0.0395	0.0239	0.8261
cis-9 C17:1	0.25	0.20	0.19	0.15	0.0177	0.0001	0.3938
Σ odd linear-chain FAs	2.84	2.56	2.04	1.83	0.1355	0.0002	0.7308
cis-even-chain monounsaturated FAs							
cis-9 C14:1	0.89	0.69	0.61	0.50	0.0975	0.0019	0.4398
cis-9 C16:1	1.28	0.97	0.62	0.76	0.1514	0.0090	0.0994
cis-9 C18:1	18.92	20.83	23.52	23.47	1.3563	0.0006	0.1327
cis-11 C18:1	0.96	1.01	1.20	1.22	0.0504	0.0005	0.5771
cis-12 C18:1	0.30	0.45	0.66	0.73	0.0545	<0.0001	0.1160
cis-13 C18:1	0.063	0.099	0.117	0.113	0.0108	0.0004	0.0082
trans-C18:1 FAs							
trans-4 C18:1	0.013	0.030	0.046	0.042	0.0046	0.0003	0.0111
trans-5 C18:1	0.044	0.024	0.033	0.143	0.0469	0.1917	0.2059
trans 6-8 C18:1	0.21	0.39	0.53	0.62	0.0455	<0.0001	0.0598
trans-9 C18:1	0.29	0.46	0.57	0.66	0.0433	<0.0001	0.1848
trans-10 C18:1	0.39	0.68	0.79	1.01	0.1133	0.0010	0.6419
trans-11 C18:1	2.16	4.20	7.37	9.76	1.1537	<0.0001	0.6619
trans-12 C18:1	0.32	0.62	0.86	1.06	0.0686	<0.0001	0.1569
trans 13-14 C18:1	0.50	0.83	0.84	1.21	0.1249	0.0002	0.7941
Σ *trans*-C18:1	3.92	7.23	11.03	14.48	1.4118	<0.0001	0.8599
Conjugated linoleic acid (CLA) isomers							
cis-9, *trans*-11 CLA	0.98	1.82	2.98	3.73	0.3971	<0.0001	0.6837
trans-9, *cis*-11 CLA	0.042	0.060	0.057	0.066	0.0076	0.0112	0.2892
trans-10, *cis*-12 CLA	0.010	0.028	0.029	0.044	0.0051	0.0009	0.8033
Long-chain polyunsaturated FAs							
cis-9, *cis*-12 C18:2 (ω-6)	1.49	1.51	1.66	1.67	0.1387	0.0023	0.7513
cis-9, *cis*-12, *cis*-15 C18:3 (ω-3)	0.22	0.20	0.22	0.19	0.0205	0.2930	0.4627

continue

continuation

Ratios between FAs							
C18:0/*trans*-11 C18:1	5.63	3.50	2.10	1.50	0.5215	<0.0001	0.0255
C18:0/*trans*-10 C18:1	33.14	20.16	20.17	15.12	4.1679	0.0053	0.4009
trans-11 C18:1/*trans*-10 C18:1	5.83	6.07	9.98	10.08	1.3434	0.0139	0.9205

The mechanisms responsible for the inhibitory effects on milk fat synthesis triggered by *trans*-10, *cis*-12 CLA or by any other FA (*e.g.*, *trans*-9, *cis*-11 CLA and *trans*-10 C18:1) are not clearly elucidated. In general, the mechanisms are associated with the reduction of mRNA abundance and the expression of lipogenic genes (*e.g.*, *ACAC, FASN, SREPB-1*), which encode transcription factors (*e.g.*, SREPB-1) and key enzymes (*e.g.*, acetyl-CoA carboxylase, FA synthetase) of *de novo* FA synthesis in the mammary gland (BERNARD et al., 2008; KADEGOWDA et al., 2009; SHINGFIELD et al., 2010). It should be noted that *de novo* FA synthesis is responsible for 100% of the linear even-chain saturated FAs C4:0 to C12:0, approximately 95% of the myristic acid, and 50% of the palmitic acid secreted in the milk (KLIEM, SHINGFIELD, 2016).

The high negative correlation between the milk contents of *trans*-10, *cis*-12 CLA and the sum of the concentrations of C4:0 to C16:0 FAs (r = -0.83; P<0.0001) can be considered indicative that this CLA isomer inhibited lipogenesis in the mammary gland via reduced *de novo* synthesis (BERNARD et al., 2008). Thus, in response to the inclusion of SO in the diet, the strong reduction (P<0.0001) in the contents of *de novo*-synthesized C4:0 to C16:0 FAs (Table 7), the sum of which showed a positive correlation with the milk fat content (r = 0.63, P=0.0096), contributed significantly to this depression. Another hypothesis to explain the reduction in the contents of *de novo*-synthesized FAs (Table 6) concerns the preferential incorporation of mono- and polyunsaturated-FAs into triglycerides of milk fat (SHINGFIELD et al., 2010). This hypothesis is supported by the negative correlations (r = -0.52 to -0.97, P<0.05) between the sum of the milk contents of *de novo*-synthesized FAs with those of several C18-unsaturated FAs (*e.g.*, rumenic; linoleic; *trans*-9, *cis*-11 CLA; *trans*-9 C18:1; *trans*-10 C18:1; vaccenic; *trans*-12 C18:1; oleic; *cis*-11 C18:1, *cis*-12 C18:1 and others). The reduction in the milk contents of *de novo*-synthesized FAs in response to supplementation of diets with free vegetable oils is also well documented in the literature (EIFERT et al., 2006; RIBEIRO et al., 2014; LOPES et al., 2015). Comparing the control ration with the 4.5% SO ration, the reductions (P<0.0001) in the milk contents of lauric, myristic and palmitic acids were, respectively, 111%, 85% and 59%.

Table 7. Indices of nutritional quality of milk fat from Holstein x Gyr dairy cows fed chopped elephantgrass-based diets containing increasing levels of soybean oil (SO).

Index	SO levels (% of diet DM)				Standard error of the mean	P-value	
	0.0	1.5	3.0	4.5		Linear	Quadratic
Atherogenicity index	3.88	2.97	2.05	1.81	0.3760	<0.0001	0.0813
Thrombogenicity index	4.83	4.03	3.13	3.00	0.4206	<0.0001	0.0676
Hypo/hypercolesterolemic fatty acids ratio	0.45	0.60	0.81	0.96	0.1053	0.0006	0.9999
ω-6/ω-3 fatty acids ratio	6.95	7.45	7.72	8.80	0.4033	0.0093	0.4359

On the other hand, there was a linear increase (P=0.0006) in the content of oleic acid in the milk (Table 6) in response to the lipid supplementation; in relation to the control treatment, the oleic acid content in the milk of the cows that received 4.5% SO increased by 24%. The production of milk fat with a higher concentration of oleic acid, which is associated with the reduction of LDL-cholesterol, and lower milk contents of lauric, myristic and palmitic acids, which are considered to be hypercholesterolemic, is desirable for human health due to their association with reducing the risk of cardiovascular disease (FAO, 2010). The h/H FA ratio, which presented a linear increase (P=0.0006) in response to the inclusion of SO in the diet, summarizes these results (Table 7). The trend of a linear reduction (P=0.0741) in the molar ratio of acetate in the rumen and linear increase in the molar ratio of butyrate (P=0.0004) in response to the inclusion of SO in the diet (Table 2) may also have contributed to the modulation of *de novo* FA synthesis in the mammary gland (BERNARD et al., 2008; SHINGFIELD, et al., 2010), since acetate and β-hydroxybutyrate are substrates for lipogenesis (BERNARD et al., 2008; SHINGFIELD et al., 2010).

Supplementation with SO promoted linear decreases (P<0.05) in the milk contents of linear even-chain saturated FAs (LECSFAs) (Table 6), which originate from the digestion and absorption of lipids synthesized by the ruminal bacteria, and the *de novo* synthesis of C13:0, C15:0 and C17:0 FAs, produced by the mammary gland with propionyl-CoA as a primer (KLIEM; SHINGFIELD, 2016). The lower milk LECSFA content in response to SO inclusion may be considered indicative of reduced activity and growth of bacterial communities as a consequence of adaptive changes in the ruminal environment, with a probable higher negative impact on the population of the cellulolytic strains, which are more vulnerable to the bacteriostatic effects of polyunsaturated-FAs (VLAEMINCK et al., 2006).

These results corroborate the linear reduction observed in the NDF ED (P=0.0358; Table 3) and, consequently, in the molar ratio of acetate (P=0.0741; Table 2). Moreover, the substitution of non-fibrous carbohydrates of the dietary ingredients by EE from the SO already promotes a decrease in fermentable substrate (RODRIGUES et al., 2017), limiting the development of the ruminal microbiota, which was indicated in the present study by the linear reduction in the excretion of purine derivatives (P=0.0127; Table 4). Corroborating this, negative correlations (P<0.05) of -0.69 and -0.59, were observed between the milk contents of the majority of the LECSFAs (*i.e.*, C15:0 and C17:0) with EE intake. On the other hand, the positive correlations (P<0.01) found between the C4:0 to C16:0 FAs and the C15:0 (r = 0.68) and C17:0 (r = 0.88) FAs are also indicative of the negative impact of SO inclusion on *de novo* FA synthesis in the mammary gland. Ruminal biohydrogenation is a detoxification mechanism performed by cellulolytic bacteria to prevent the bacteriostatic effects of polyunsaturated-FAs (MAIA et al., 2010). In the present work, there was impressive ruminal biohydrogenation of linoleic acid in response to the inclusion of SO in the diet. This result is confirmed by the linear increments in the milk contents of *cis*-C18:1, *trans*-C18:1, and the CLA isomers (Table 6). All of these FAs are important intermediates of ruminal biohydrogenation of linoleic acid (SHINGFIELD et al., 2008, 2010), which was quantitatively the most-consumed polyunsaturated-FA in cows receiving diets with SO. In addition, due to ruminal biohydrogenation, there was a linear increase (P=0.0191) in the stearic acid content in milk (Table 6). However, comparing the control ration with the 4.5% SO ration, this increase was only 28%, which indicates that the inclusion of SO reduced, at least in part, the extent of ruminal biohydrogenation. This result, which is associated with the linear increase (P=0.0023) observed in the milk content of linoleic acid (Table 6), may indicate that the capacity of ruminal biohydrogenation of this polyunsaturated-FA was partially impaired,

increasing its escape from the rumen and its availability for uptake by the mammary gland. This explains the greater secretion of linoleic acid in the milk of the cows that consumed the diets with SO. The main route of ruminal biohydrogenation of linoleic acid is carried out by bacteria of the species *Butyrivibrio fibrisolvens* with formation of rumenic and vaccenic acids (McKAIN et al., 2010). However, the high input of linoleic acid in the rumen can trigger the need to adapt the ruminal microbiota for the execution of biohydrogenation by an alternative route (ZENED et al., 2013). In this sense, McKain et al. (2010) demonstrated *in vitro* that the bacterium *Propionibacterium acnes* does not metabolize geometric isomers of CLA but is specialized in isomerizing linoleic acid to *trans*-10, *cis*-12 CLA. In addition, these authors also reported that *B. fibrisolvens* JW11 metabolized *trans*-10, *cis*-12 CLA to *trans*-10 C18:1, *trans*-12 C18:1 and *cis*-12 C18:1; corroborating this, there were positive correlations (P<0.01) between the milk contents of *trans*-10, *cis*-12 CLA, with these three monounsaturated isomers, respectively, of 0.77, 0.86 and 0.71. Comparing the control ration with the 4.5% SO ration, the significant increases of 281% and 340% observed in the milk contents of rumenic and *trans*-10, *cis*-12 CLA (Table 6), respectively, demonstrate that the two biohydrogenation pathways of linoleic acid were concomitantly carried out by the ruminal microbiota. In addition, assuming that the linear increase (P=0.0009) in the milk content of *trans*-10, *cis*-12 CLA (Table 6), was a reflection of its concentration in the rumen of cows, it is possible that this may have also contributed to the reduction of the population of *Butyrivibrio proteoclasticus*. In this sense, McKain et al. (2010) observed that the P-18 strain of this bacterium did not grow in medium containing this CLA isomer. According to these authors, *B. proteoclasticus* performs the final step of biohydrogenation, converting vaccenic and *trans*-10 C18:1 acids into stearic in the rumen. Therefore, the reduction of its population in the rumen is related to the accumulation of *trans*-C18:1 FAs at the expense of stearic acid. The reductions (P<0.01) observed in stearic/vaccenic and stearic/*trans*-10 C18:1 ratios in the milk of cows receiving SO (Table 6) can be considered indicative of a differential sensitivity of ruminal cellulolytic bacteria species to the bacteriostatic effects of linoleic acid (VLAEMINCK et al., 2006). This corroborates the results of Paillard et al. (2007), who reported that *B. proteoclasticus* is more vulnerable to the toxic effects of polyunsaturated-FAs than *B. fibrisolvens*.

The major *trans*-monounsaturated isomers, intermediates of the ruminal biohydrogenation routes of linoleic acid, are the elaidic (*trans*-9 C18:1), *trans*-10 C18:1 and vaccenic FAs (SHINGFIELD et al., 2010). Comparing the control ration with the 4.5% SO ration, the observed increases in the milk contents of these three FAs were 128%, 159% and 352% (Table 6). The elaidic and *trans*-10 C18:1 FAs have been associated with deleterious effects on cardiovascular health (ALMEIDA et al., 2014); therefore, increasing their milk contents is not desirable for human nutrition. However, while SO inclusion promoted a linear increase (P<0.01) in the milk concentrations of the elaidic and *trans*-10 C18:1 FAs (Table 6) between treatments, they are located in the range of values, respectively, of 0.33 to 0.73 g 100 g^{-1} FAs and 0.51 to 18.62 g 100 g^{-1} FAs, compiled by Kliem and Shingfield (2016) in studies with the inclusion of vegetable oils in diets supplied to cows.

The linear increase (P=0.0139) in the vaccenic/*trans*-10 C18:1 FA ratio (Table 6) shows that as SO was increasingly included in the diet, the route of formation of vaccenic acid became prevalent over that of *trans*-10 C18:1. In terms of human health, this result is particularly important, since vaccenic acid, which was the major isomer among the *trans*-C18:1 FAs (Table 6), is the precursor for the synthesis of 70% to 95% of all rumenic acid secreted in bovine milk (KLIEM; SHINGFELD, 2016). Therefore, diets that promote an increase in

the supply of vaccenic acid from the rumen to the mammary gland generally result in higher levels of rumenic acid in milk. In addition, 19% of the consumed vaccenic acid is converted to rumenic acid in human tissues (TURPEINEN et al., 2002). The regression of rumenic *versus* vaccenic acids in milk (g 100 g^{-1} FAs) demonstrates the close association between these FAs ($\hat{y}$ = 0.33603*x + 0.4027; r^2 = 0.88; P<0.0001).

The contents of vaccenic and rumenic acids in milk obtained with the inclusion of 4.5% SO (Table 6) were quite impressive and were superior to the respective ranges of 3.11 to 7.97 g 100 g^{-1} FAs and 1.22 to 3.24 g 100 g^{-1} FAs, compiled by Lopes et al. (2015) of studies of cows fed diets based on chopped elephantgrass and inclusion of 1.3% to 4.5% sunflower oil. These values can be considered high when compared to the milk of cows receiving typical confinement diets, based on conserved forages and concentrates, illustrating the potential of tropical grasses in the production of milk enriched with rumenic and vaccenic acids (LOPES et al., 2015). The high concentrations of rumenic acid in the milk of cows receiving SO were potentiated by the linear increase (P=0.0645) observed in the stearoyl-CoA desaturase (SCD) activity index for the rumenic/vaccenic pair (data not shown). It should be noted that there was no correlation (P>0.05) between the SCD activity on the rumenic/vaccenic pair and the milk contents of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA. In a study with bovine mammary epithelium, Kadegowda et al. (2009) reported that these two FAs reduced the expression of the SCD enzyme by 100% and 357%. For the myristic/*cis*-9 C14:1, palmitic/*cis*-9 C16:1 and stearic/oleic pairs, there was no effect (P>0.05) of treatments on the SCD activity (data not shown). The absence of an effect of SO on the index of SCD activity on the oleic/stearic pair indicates the importance of the contribution of oleic acid captured by the mammary gland to its secretion in milk. The esterification of oleic acid in the *sn*-3 position of triacylglycerol constitutes an important mechanism of control of the melting point and the fluidity of the milk fat (BERNARD et al., 2008).

The inclusion of SO in the diet improved the nutritional quality of milk fat, since there was a linear reduction (P<0.0001) in AI and TI and an increase (P=0.0006) in the h/H FA ratio (Table 7). This can be attributed mainly to the decrease in the milk contents of the hypercholesterolemic lauric, myristic and palmitic FAs (FAO, 2010), the concomitant increase in oleic acid content, and specifically considering TI, also due to the increase in stearic acid content in milk (Table 6). The increase (P=0.0093) in the ω-6/ω-3 FA ratio in response to SO supplementation was a consequence of the linear increase (P=0.0023) in the milk linoleic acid content (ω-6), since there was no effect (P>0.05) of the treatments on the α-linolenic acid content (ω-3) in milk (Table 7). However, the ω-6/ω-3 FA ratio is not a useful index as a metric for dietary recommendations aimed at reducing the risk of cardiovascular disease (FAO, 2010).

Conclusions

The inclusion of soybean oil in diets based on chopped elephantgrass reduced fiber degradability but did not alter the dry matter intake. SO improved the nutritional quality of milk fat with an increase in the contents of fatty acids beneficial to human health and a reduction in hypercholesterolemic fatty acid milk content.

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