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Ruminal parameters and fatty acid composition of omasal digesta and milk in cows fed sugarcane-based diets supplemented with sunflower oil

Parâmetros ruminais e perfil de ácidos graxos da digesta omasal e do leite de vacas alimentadas com dietas à base de cana de açúcar suplementadas com óleo de girassol

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Highlights:
Intake, digestibility and rumen degradability of DM and fiber were unchanged by sunflower oil.
Moderate sunflower oil levels promoted milk fat depression in cows receiving sugarcane-based diets.
Nutritional quality of milk fat was improved by sunflower oil in sugarcane-based diets.

Abstract

This study evaluates the intake and digestion of nutrients, parameters of rumen fermentation and degradation, omasal digesta and milk fatty acid composition, productive performance, and the concentration of serum metabolites in cows fed 600 g kg^{-1} sugarcane-based diets containing 0 (control), 15, 30, and 45 g kg^{-1} sunflower oil (SO) on a dry matter (DM) basis. Four rumen-cannulated Holstein x Gyr cows yielding 15±5 kg day^{-1} with 110±10 days in milk were allocated in a 4 x 4 Latin square design. Data were analyzed using mixed models, and significant differences were declared at $P<0.05$. There was no effect of SO on the intake and apparent digestibility of DM, crude protein, neutral detergent fiber (NDF) and nonfibrous carbohydrates, but there was a linear increase in the intake and digestibility of ether extract. Dietary SO levels did not alter the ruminal degradability parameters for DM and NDF, rumen pH and contents of ammonia N, acetate, propionate and volatile fatty acids. Milk fat content and yield were linearly decreased, whereas a linear increase in milk protein content was observed in response to increasing levels of SO, but with no effect on milk yield. Linear reductions in palmitic and α-linolenic acid contents, a linear increase in *trans*-10 C18:1 and elaidic acids, and a quadratic effect on vaccenic and rumenic acids were observed in omasal digesta of cows fed increasing levels of SO. Overall, up to 45 g kg^{-1} SO can be included on DM of chopped sugarcane-based diets without reducing consumption, apparent digestibility and rumen degradability of DM and fiber. Supplementing chopped sugarcane-based diets with 30 to 45 g kg^{-1} SO (DM basis) promotes milk fat depression due to the inhibition of mammary lipogenesis by specific rumen-derived fatty acid intermediates of the biohydrogenation of unsaturated C18 fatty acids. The inclusion of 15 to 45 g kg^{-1} SO in chopped sugarcane-based diets improves the nutritional quality of milk fat, with increases in the levels of oleic, vaccenic and rumenic acids, beneficial to human health, and a reduction in the levels of the hypercholesterolemic lauric, myristic and palmitic acids.

Key words: Conjugated linoleic acid. Rumenic acid. *Saccharum officinarum*. Vaccenic acid.

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Resumo

O objetivo deste estudo foi avaliar o consumo e a digestão dos nutrientes, os parâmetros de fermentação e degradação ruminal, o perfil de ácidos graxos da digesta omasal e do leite, o desempenho produtivo e as concentrações séricas de metabólitos em vacas alimentadas com dietas à base de 600 g kg^{-1} de cana de açúcar com 0 (controle), 15, 30 e 45 g kg^{-1} de óleo de girassol (OG) na base da matéria seca (MS). Foram utilizadas quatro vacas Holandês x Gir com 110±10 dias em lactação, produzindo 15±5 kg dia^{-1} de leite, canuladas no rúmen, e alocadas em delineamento Quadrado Latino 4 x 4. Os resultados foram analisados por modelos mistos e efeitos considerados significativos quando $P<0,05$. Não houve efeito do OG no consumo e digestibilidade aparente da MS, proteína bruta, fibra em detergente neutro (FDN) e carboidratos não fibrosos, mas houve aumento linear no consumo e digestibilidade aparente do extrato etéreo. Não houve efeito do OG sobre os parâmetros de degradabilidade da MS e FDN, sobre o pH do rúmen nem sobre as concentrações ruminais de N amoniacal, acetato, propionato e ácidos graxos voláteis totais. O OG promoveu redução linear no teor e na produção de gordura do leite, aumento linear no teor de proteína do leite, mas não houve efeito sobre a produção de leite. Na digesta omasal, o OG promoveu reduções lineares nos teores dos ácidos palmítico e α-linolênico, aumentos lineares nos teores dos ácidos elaídico e C18:1 *trans*-10, e efeito quadrático nos teores dos ácidos vacênico e rumênico. Concluindo, até 45 g kg^{-1} de OG pode ser incluído na MS de dietas à base de cana de açúcar picada, sem reduzir o consumo, a digestibilidade aparente a degradabilidade ruminal da MS e da fibra. A suplementação da MS de dietas à base de cana de açúcar picada com 30 a 45 g kg^{-1} de óleo de girassol promove redução no teor de gordura do leite, devido à inibição da lipogênese mamária por específicos ácidos graxos formados no rúmen a partir da bio-hidrogenação de ácidos graxos insaturados C18. A inclusão de 15 a 45 g kg^{-1} de óleo de girassol em dietas à base de cana de açúcar picada melhora a qualidade nutricional da gordura do leite, com aumentos nos teores dos ácidos oleico, vacênico e rumênico, que são benéficos para a saúde humana, e redução nos teores dos ácidos hipercolesterolêmicos láurico, mirístico e palmítico.

Palavras-chave: Ácido linoleico conjugado. Ácido rumênico. Ácido vacênico. *Saccharum officinarum*.

Introduction

Sugarcane (*Saccharum officinarum* L.) is a roughage traditionally used in Brazilian dairy farms during the seasonal period of growth in tropical grasses(Campos, Lopes, Pereira, Machado, & Tomich, 2017). However, besides the low crude protein (CP) content, sugarcane presents other nutritional limitations that restrict its use as an exclusive forage in the diets of high-producing dairy cows such as a high content of rumen-undegradable neutral detergent fiber (NDF) and a low ruminal digestion rate of the potentially degradable NDF (S. A. Santos et al., 2011; R. C. O. Ribeiro et al., 2015). These fractions induces a marked effect on rumen fill and reduces the dry matter (DM) intake, negatively impacting the productive performance of cows fed sugarcane-based diets (S. A. Santos et al., 2011).

Supplementation with vegetable oils represents an alternative nutritional strategy to augment energy density without increasing the nonfiber carbohydrate (NFC) content in sugarcane-based diets (Rodrigues et al., 2017, 2019). Notably, vegetable oils, including sunflower oil (SO), are rapidly released in the rumen and may have deleterious effects on the resident microbiota and fiber digestion and may affect milk fat content and fatty acid (FA) composition (Rico, Holloway, & Harvatine, 2015a).

In a companion study performed concomitantly with the present trial using the same treatments, Souza et al. (2019) demonstrated that up to 45 g kg^{-1} SO in a sugarcane-based diet improved the nutritional quality of milk fat as a result of the increased content of oleic (*cis*-9 C18:1), vaccenic (*trans*-11 C18:1), and rumenic (*cis*-9, *trans*-11 CLA) FAs, all of which are beneficial to human health. In addition to these improvements, there was a decrease in the content of hypercholesterolemic lauric (C12:0), myristic (C14:0), and palmitic (C16:0) FAs. However, SO

linearly reduced the milk fat content and yield, and there was an increase in *trans*-10 C18:1 and elaidic acid (*trans*-9 C18:1), both of which are associated with deleterious effects on cardiovascular health (Vahmani, Meadus, Duff, Rolland, & Dugan, 2017). This work complements the study of Souza et al. (2019) and presents results contributing to a better understanding of the association between nutrient metabolism and production responses in dairy cows fed sugarcane-based diets containing increasing levels of SO.

The aim of this study was to evaluate the intake and digestion of nutrients, ruminal fermentation and degradation parameters, omasal digesta and milk fatty acid composition, productive performance, and serum metabolites in Holstein x Gyr cows fed sugarcane-based diets supplemented with sunflower oil.

Materials and Methods

The study was carried out at Embrapa Dairy Cattle (Coronel Pacheco, MG, Brazil) from July to December 2009. All experimental procedures with animals were conducted according to Embrapa Dairy Cattle guidelines for animal care and use in research. Four multiparous Holstein x Gyr cows (500±39 kg) with 110±10 days in milk and producing 15±5 kg day^{-1} of milk at the beginning of the study were used. A 4 x 4 Latin square (LS) experimental design with 19-day periods was used, the first 10 days for diet adaptation and the remaining nine days for sampling and data recording.

The experimental treatments were diets based on chopped sugarcane with 0 (control), 15, 30, and 45 g kg^{-1} sunflower oil (SO) provided on a DM basis. The sugarcane variety RB-739735, averaging 18±4°brix, was used. Diets were formulated to be isoproteic and isofibrous (Table 1) and to meet the requirements of cows 500 kg in body weight (BW) producing 17 kg day^{-1} of milk with 0.3 kg day^{-1} of weight gain, in accordance with the recommendations of the National Research Council [NRC] (2001). A more detailed description of the ingredients and chemical composition of the experimental diets was previously described (Souza et al., 2019). Briefly, the diets were formulated at a fixed forage:concentrate ratio of 60:40 (DM basis), and the concentrates were formulated with corn meal, citrus pulp, urea + ammonium sulfate (9:1), mineral and vitamin supplement, and with SO partially replacing the energy from corn meal and citrus pulp.

Table 1
Chemical composition of the experimental diets on a dry matter (DM) basis*

Item	Sunflower oil levels (g kg^{-1} DM)			
	0	15	30	45
Chemical composition (g kg^{-1} DM)				
Crude protein	129	128	127	126
Ether extract	18	32	45	59
Neutral detergent fiber corrected for ash and protein	311	312	315	313
Nonfibrous carbohydrate corrected for ash and protein	479	445	411	388
Fatty acid composition (g kg^{-1} DM)				
cis-9 C18:1 (oleic acid)	4.21	7.93	10.66	12.14
cis-9, *cis*-12 C18:2 (linoleic acid)	7.23	17.22	26.25	31.73
cis-9, *cis*-12, *cis*-15 C18:3 (α-linolenic acid)	0.73	0.95	1.23	1.35

***Source**: Adapted from Souza et al. (2019).

The cows were collectively lodged in a free stall equipped with electronic troughs (American Calan Inc., Northwood, NH, USA) that allowed individual control of feed intake. Feed was supplied *ad libitum* (10% of orts) as a TMR once a day (08h00) using a mixer/dispenser vehicle (Data Ranger; American Calan Inc.). The sugarcane was chopped daily, and the concentrates with SO were prepared weekly to avoid lipid peroxidation.

Samples of sugarcane and concentrates were collected in each LS period. Samples of individual orts were also collected and pooled per cow. Fecal samples were collected every 26 h from 08h00 on day 11 to day 16 of each LS period, and pooled per cow. All samples were stored (-20°C), and at the end of the trial, they were thawed, predried (55°C, 72-96 h), milled (1 mm) and analyzed in the Laboratory of Food Analysis of Embrapa Dairy Cattle (Juiz de Fora, MG, Brazil) for DM (at 105°C), CP, ether extract (EE), mineral matter, NDF corrected for ash and protein (NDF_{ap}), NFC corrected for ash and protein (NFC_{ap}) and lignin content, according to Detmann et al. (2012). Samples of chopped sugarcane collected in each LS period and samples of each ingredient of the concentrates, besides the SO, were analyzed for FA composition and the results presented in the companion paper by Souza et al. (2019). Samples of omasal digesta (400 g) were collected using equipment and procedures described by Leão (2002), at 11h00 and 23h00 on day 12, at 15h00 on day 13, at 03h00 and 19h00 on day 14, and at 07h00 on day 15 of each LS period, pooled per cow, stored (-20°C), lyophilized, ground (1 mm), and analyzed for FA composition as described by Shingfield et al. (2003).

Indigestible neutral detergent fiber, quantified via 264 h of ruminal *in situ* incubation using F57 filter bags (ANKOM Technology, Macedon, NY, USA), was used as an internal marker to estimate digestibility.

The ruminal parameters were evaluated on the 10th and 11th days of each LS period. Samples of ruminal fluid were collected before (time 0) and 2, 4, 6, 8, 10, 12, 18 (10th day) and 24 hours (11th day) after the provision of the ration. They were filtered using gauze and the pH measured using a digital potentiometer. Two 10-mL aliquots were added to flasks with 0.4 mL of sulfuric acid (50% v/v, subsample 1) or 2 mL of metaphosphoric acid (25% v/v, subsample 2) and frozen. Subsample 1 was analyzed for ammonia N content ($N\text{-}NH_3$) 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 concentration (mmol 100 mL^{-1}) of volatile fatty acids (VFAs) using a GC model 7820A (Agilent Technologies, Inc., Santa Clara, CA, USA) with a flame ionization detector 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 degradability (Nocek, 1988), a predried (55°C, 72 h) and milled (5 mm) sample of sugarcane was used, conditioned in nylon bags (10 x 20 cm, 50 µ porosity, 10-20 mg of sample per cm^2) and 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 6, 12, 24, 48, 72 and 96 hours later, and frozen. Subsequently, they were washed, predried (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 a nonlinear model (Tomich & Sampaio, 2004) using the NLIN procedure of SAS. Curves were generated for each cow in each LS period. The effective degradabilities were calculated (Ørskov & McDonald, 1979) based on the passage rate of solids in the rumen (kp, % $hour^{-1}$) estimated by an equation described in NRC (2001): kp = 3.054 + (0.614*BW_DMI), where BW_DMI is the DM intake (kg 100 kg^{-1} of body weight).

Cows were milked twice daily (06h00 and 14h00), and their milk yields were recorded from

the 14th to the 19th days of each LS period. Aliquots of milk from each milking (2/3 at morning milking + 1/3 at afternoon milking) were collected to compose individual samples (30 mL), stored in bottles with a bronopol preservative for analysis of protein, fat, lactose and total solids by medium infrared spectrometry (Bentley 2300; Bentley Instruments, Inc., Chaska, MN, USA), and for analysis of milk urea nitrogen (MUN) at the Embrapa Dairy Cattle (Juiz de Fora, MG). Milk samples for FA analyses were collected on the 19th day of each LS period and analyzed using the GC according to the procedures described by C. G. S. Ribeiro, Lopes, Gama, Rodriguez and Morenz (2018).

The nutritional quality of milk fat was evaluated by the atherogenicity (AI) and thrombogenicity (TI) indices, omega-6 and omega-3 FA ratio (ω-6/ω-3 FA ratio) and hypo- and hypercholesterolemic FA ratio (h/H FA ratio), calculated according to C. G. S. Ribeiro et al. (2018).

Blood samples were collected on the 17th day of each LS period via coccygeal venipuncture and stored in test tubes (Vacutainer; Becton-Dickinson, Rutherford, NJ, USA) containing EDTA anticoagulant. The samples were immediately centrifuged at 3,000 x *g* for 15 min, and plasma (2 mL) was stored in tubes (-20°C) until analysis. The serum contents of nonesterified FA (NEFA), glucose, cholesterol, triglycerides and urea nitrogen were analyzed using commercial kits as described by Rodrigues et al. (2017).

The results were analyzed using mixed models using the MIXED procedure of SAS. The ruminal fermentation parameters were analyzed as repeated measures in time, with the following considered to be fixed effects: treatment (level of SO in the diet), sampling time and the interaction between these factors; and the following were considered 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, and significant differences were declared at $P<0.05$. Regression equations between specific variables were adjusted using the REG procedure, and Pearson correlations were calculated using the CORR procedure of SAS.

Results

There was no effect ($P>0.05$) of SO on the intake of DM and NDF_{ap} (g day^{-1} and kg 100 kg^{-1} of BW), CP, NFC_{ap} and total digestible nutrients (TDN), but there was a linear increase ($P<0.0001$) in the intakes of EE and α-linolenic acid (Table 2). SO promoted quadratic effects ($P<0.05$) on the palmitic, stearic (C18:0), oleic and linoleic acid intakes (Table 2); maximum values of 79, 28, 177 and 468 g day^{-1} were estimated when 35.9, 43.2, 42.5 and 51.2 g kg^{-1} SO was added to the diet, respectively. There was no effect ($P>0.05$) of SO on the apparent digestibility of DM, CP, NDF_{ap} and NFC_{ap}, but SO promoted a linear increase in the apparent digestibility of EE ($P=0.0057$) and on TDN ($P=0.0032$) (Table 2).

There was no effect ($P>0.05$) of SO on the rate passage of solids in the rumen and on the ruminal degradability parameters for DM and NDF (Table 3). The treatment*sampling time interaction was not significant ($P>0.05$) for any of the ruminal parameters (Table 4). No effect was observed ($P>0.05$) of the treatments on rumen pH, acetate:propionate ratio, and on rumen contents of $N\text{-}NH_3$, acetate, propionate and total VFAs, but SO promoted a linear reduction ($P=0.0254$) in the rumen content of butyrate (Table 4). The sampling time promoted a quadratic effect on rumen pH and total VFA content. The minimum rumen pH value was 5.8, and the maximum value of rumen total VFA content was 72.6 mmol 100 mL^{-1}, estimated to occur at 11.3 h and 11.9 h after the provision of the ration, respectively (Figure 1).

Table 2
Intake and apparent digestibility of nutrients in Holstein x Gyr cows fed sugarcane-based diets containing increasing levels of sunflower oil (SO)

Item	SO levels (g kg^{-1} DM)				SEM	P-value	
	0	15	30	45		Linear	Quadratic
Nutrient intake (kg day^{-1})							
Dry matter (DM)	14.6	15.5	16.1	14.4	1.2797	0.9672	0.1131
Crude protein (CP)	1.85	1.96	2.00	1.80	0.1419	0.7664	0.1501
Ether extract (EE)	0.258	0.508	0.733	0.878	0.0492	<0.0001	0.2357
NDF_{ap} [a]	5.00	5.42	5.79	5.10	0.5114	0.6213	0.1041
NFC_{ap} [b]	7.20	7.23	7.12	6.26	0.6426	0.0599	0.1643
TDN[c]	10.8	11.6	12.1	11.4	1.0905	0.2828	0.1480
Fatty acid intake (g day^{-1})							
Palmitic acid	42.9	63.9	79.8	75.4	5.7661	0.0004	0.0125
Stearic acid	8.2	18.0	27.3	27.4	1.9937	<0.0001	0.0107
Oleic acid	61.3	123.0	171.4	174.8	12.8590	<0.0001	0.0138
Linoleic acid	105.2	267.0	422.2	457.2	32.9181	<0.0001	0.0300
α-linolenic acid	10.6	14.6	19.8	19.4	1.4927	<0.0001	0.0512
Nutrient intake (kg 100 kg^{-1} of body weight)							
DM	3.03	3.23	3.42	3.04	0.2382	0.7827	0.1247
NDF_{ap} [a]	1.04	1.13	1.20	1.08	0.1000	0.5418	0.1334
Apparent digestibility (%)							
DM	71.3	70.3	69.4	71.4	2.3737	0.8939	0.1119
CP	69.1	68.1	69.7	70.6	2.8639	0.3616	0.5409
EE	86.8	95.3	93.7	97.2	1.6600	0.0057	0.1691
NDF_{ap} [a]	42.5	42.9	42.9	46.7	4.5954	0.0745	0.2688
NFC_{ap} [b]	95.4	94.7	93.6	93.9	0.9904	0.0668	0.4397
TDN[c]	73.5	74.9	75.2	79.6	2.6619	0.0032	0.1262

[a]NDF_{ap} = neutral detergent fiber corrected for ash and protein; [b]NFC_{ap} = nonfibrous carbohydrate corrected for ash and protein; [c]TDN (Total digestible nutrients) = DCP + $DNDF_{ap}$ + $DNFC_{ap}$ + 2.25*DEE, where DCP, $DNDF_{ap}$, $DNFC_{ap}$ and DEE = digestible CP, NDF_{ap}, NFC_{ap} and EE, respectively (Weiss, 1999).

Table 3
Rate passage of solids in the rumen and the dry matter (DM) and neutral detergent fiber (NDF) degradability parameters of chopped sugarcane in Holstein x Gyr cows fed sugarcane-based diets containing increasing levels of sunflower oil (SO)

Item	SO levels (g kg^{-1} DM)				SEM	P-value	
	0	15	30	45		Linear	Quadratic
Rate passage of solids in the rumen[a]	4.91	5.04	5.15	4.92	0.1463	0.7827	0.1247
Ruminal DM degradability							
Potential degradability (%)	67.9	68.5	66.5	68.7	0.8695	0.9400	0.3639
Degradation rate (% hour^{-1})	2.26	2.19	2.43	2.11	0.4105	0.7779	0.4813
Effective degradability (%)[b]	25.1	25.2	25.4	24.4	2.3445	0.6119	0.5561
Ruminal NDF degradability							
Potential degradability (%)	48.6	48.1	46.8	51.6	2.1597	0.3242	0.1443
Degradation rate (% hour^{-1})	2.40	2.35	2.55	2.29	0.4102	0.8587	0.5536
Effective degradability (%)[b]	15.3	15.1	15.0	15.6	1.6252	0.6645	0.3473

[a]Estimated by the following equation (NRC, 2001): 3.054 + (0.614*BW_DMI). Where BW_DMI is the DM intake expressed in kg 100 kg^{-1} of body weight; [b]Calculated considering the passage rate of solids in the rumen estimated by the NRC (2001) equation for wet forage.

Table 4
Ruminal fermentation parameters in Holstein x Gyr cows fed sugarcane-based diets containing increasing levels of sunflower oil (SO)

Item	SO levels (g kg^{-1} DM)				SEM	P-value		
	0	15	30	45		Linear	Quadratic	Trat*Time[a]
pH	6.15	6.07	6.20	6.15	0.1675	0.6698	0.8501	0.7894
Ammonia N (mg dL^{-1})	14.99	12.03	13.46	13.22	1.9371	0.5883	0.5966	0.9661
Acetate (A)	42.41	37.07	40.36	39.75	3.3925	0.5903	0.2367	0.5960
Propionate (P)	15.59	13.41	15.29	16.09	1.3019	0.4758	0.1664	0.9314
Butyrate	10.20	9.04	8.42	9.85	0.9664	0.4113	0.0254	0.9400
VFA[b]	68.21	59.52	64.07	65.68	5.0670	0.8425	0.1456	0.8368
A: P ratio	2.91	2.91	2.80	2.70	0.1799	0.3384	0.7278	0.7326

[a]Interaction between treatment (g kg^{-1} sunflower oil in the diet) and sampling time; [b]VFA (volatile fatty acids) = Σ ruminal concentrations (mmol 100 mL^{-1}) of acetate + propionate + butyrate.

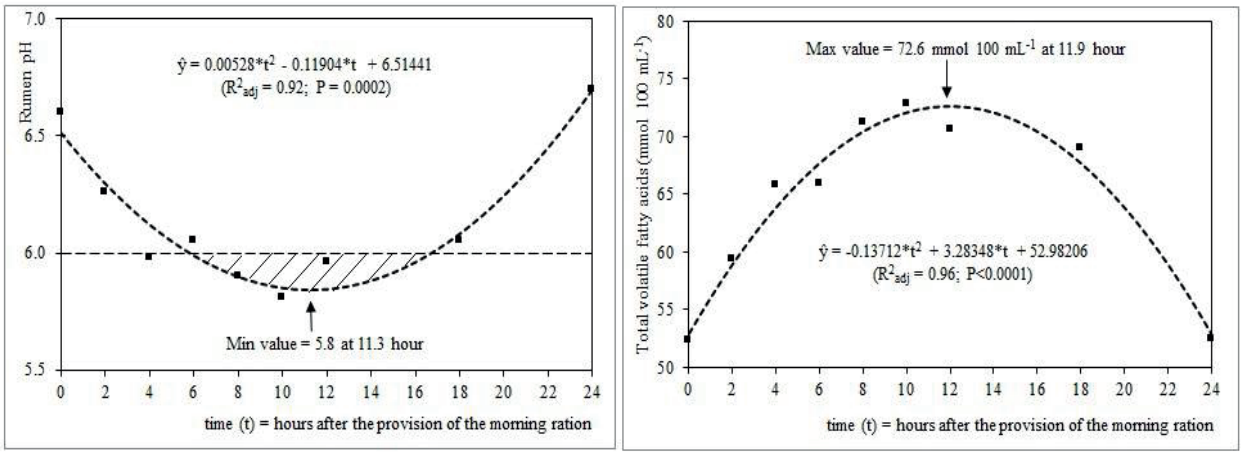

Figure 1. Effect of sampling time (t, hour) on rumen fermentation parameters in Holstein x Gyr cows fed sugarcane-based diets containing 0, 15, 30, and 45 g kg^{-1} sunflower oil on a dry matter basis.

SO promoted linear decreases in milk fat (P=0.0026) and total solids contents (P=0.0046) and a linear increase in milk protein content (P=0.0242). However, no effect (P>0.05) on MUN and milk lactose content was observed. The milk yield, fat-corrected milk yield, and milk protein, lactose and total solids yields were not influenced (P>0.05) by SO, but there was a linear reduction (P=0.0328) in milk fat yield (Table 5). SO promoted a linear decrease (P=0.0025) in the apparent transfer into milk of ingested α-linolenic acid and a quadratic effect (P<0.05) on the apparent transfer of ingested oleic and linoleic acids into milk (Table 5) with maximum values of 0.408 and 0.183 estimated for 36.8 and 35.4 g kg^{-1} SO in the ration, respectively.

There was no effect (P>0.05) of SO on the serum concentrations of glucose, NEFA, triglycerides and urea nitrogen, but there was a quadratic effect (P=0.0247) on serum cholesterol content (Table 6) with a maximum value (175.7 mg dL^{-1}) estimated when 32.8 g kg^{-1} SO was added to the diet.

SO promoted linear reductions (P<0.05) in the omasal digesta contents of palmitic and α-linolenic acids, linear increases (P<0.05) in *trans*-10 C18:1 and elaidic acid contents, and quadratic effects (P<0.05) on *de novo*-synthesized FAs C4:0 to C16:0 and on the vaccenic and rumenic acid contents (Table 7). The minimum omasal digesta content of *de novo*-synthesized FAs was 25.3 g 100 g^{-1} FA,

estimated to occur at 29.8 g kg^{-1} SO in the ration, while the maximum omasal digesta contents of vaccenic and rumenic acid were 9.03 and 1.45 g 100 g^{-1} FA, estimated to occur at 31.8 and 19.8 g kg^{-1} SO in the ration, respectively. SO promoted linear reductions (P<0.01) in the milk fat contents of the major odd- and branched-chain FAs (OBCFAs), and lauric, myristic and palmitic acids, and linear increases (P<0.05) in stearic, oleic, elaidic, *trans*-10 C18:1, *trans*-10, *cis*-12 CLA, *trans*-9, *cis*-11 CLA, vaccenic and rumenic FA contents (Tables 8 and 9). SO did not alter the milk fat linoleic and α-linolenic acid contents (Table 9). SO promoted a linear reduction (P=0.0061) in SCD activity only for rumenic/vaccenic FAs (data not shown). SO promoted linear reductions in AI and TI (P<0.01) and linear increases (P=0.001) in the h/H and ω-6:ω-3 (P=0.003) FA ratios (data not shown).

Table 5
Milk yield and composition and apparent transfer of ingested fatty acids (FA, g day^{-1}) into milk (g day^{-1}) of Holstein x Gyr cows fed sugarcane-based diets containing increasing levels of sunflower oil (SO)

Item	SO levels (g kg^{-1} DM)				SEM	P-value	
	0	15	30	45		Linear	Quadratic
Yield							
Milk (kg day^{-1})	15.1	15.6	16.1	15.1	1.1168	0.8650	0.4279
FCM (kg day^{-1})[a]	14.4	13.8	12.8	12.0	1.0201	0.1123	0.9246
Fat (g day^{-1})	556.2	503.2	424.6	397.0	46.7803	0.0328	0.7879
Protein (g day^{-1})	490.0	498.3	526.1	521.6	45.0530	0.2872	0.7934
Lactose (g day^{-1})	653.0	687.6	690.8	646.8	48.9985	0.9298	0.3367
Total solids (g day^{-1})	1,834.0	1,827.2	1,778.1	1,693,6	126.690	0.3747	0.7352
Composition (%)							
Fat	3.69	3.25	2.66	2.65	0.2400	0.0026	0.2555
Protein	3.25	3.20	3.29	3.44	0.1683	0.0242	0.0929
Lactose	4.34	4.43	4.31	4.28	0.0743	0.0717	0.1085
Total solids	12.16	11.76	11.10	11.22	0.2540	0.0046	0.1999
Milk urea N (mg dL^{-1})	12.2	13.1	10.7	11.1	1.5074	0.3333	0.8329
Apparent transfer of ingested fatty acids (FA, g day^{-1}) into milk (g day^{-1})							
Oleic acid	1.399	0.709	0.483	0.443	0.1047	0.0003	0.0207
Linoleic acid	0.102	0.035	0.025	0.018	0.0066	<0.0001	0.0035
α-Linolenic acid	0.087	0.043	0.032	0.020	0.0098	0.0025	0.1487

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

Table 6
Serum metabolites in cows fed sugarcane-based diets containing increasing levels of sunflower oil (SO)

Metabolite	SO levels (g kg^{-1} DM)				SEM	P-value	
	0	15	30	45		Linear	Quadratic
Glucose (mg dL^{-1})	52.5	52.9	58.5	56.3	2.4578	0.0569	0.4891
Nonesterified fatty acids (mmol L^{-1})	0.168	0.223	0.240	0.165	0.0705	0.9598	0.1811
Cholesterol (mg dL^{-1})	88.7	137.3	186.7	159.3	15.7748	0.0038	0.0247
Triglycerides (mg dL^{-1})	3.27	3.43	3.87	4.45	0.8382	0.3263	0.8074
Urea nitrogen (mg dL^{-1})	10.8	14.1	11.8	10.5	3.0080	0.3844	0.1224

Table 7
Selected fatty acids (FA) in omasal digesta of Holstein x Gyr cows fed sugarcane-based diets containing increasing levels of sunflower oil (SO)

Fatty acid (g 100 g^{-1} FA)	SO levels (g kg^{-1} DM)				SEM	P-value	
	0	15	30	45		Linear	Quadratic
Σ 4≤C≤10	4.15	2.54	2.60	3.09	0.4024	0.1234	0.0358
C12:0	8.54	7.13	5.57	6.65	0.7985	0.0894	0.1703
C14:0	5.23	2.82	3.25	3.18	0.6262	0.0790	0.1028
C16:0	19.53	14.62	15.00	15.10	1.2948	0.0489	0.0765
Σ 4≤C≤16	37.45	27.11	26.40	28.01	2.5504	0.0366	0.0487
C18:0	23.94	31.64	29.71	28.61	2.6787	0.3398	0.1419
iso C15:0	0.68	0.55	0.50	0.54	0.0606	0.1251	0.2042
anteiso C15:0	0.99	0.69	0.72	0.72	0.0864	0.0886	0.1364
C15:0	0.56	0.74	0.52	0.59	0.0630	0.7223	0.3968
C17:0	0.52	0.40	0.34	0.40	0.0574	0.1450	0.1583
cis-9 C18:1	11.66	10.37	9.62	8.33	1.5235	0.1656	0.9981
trans-9 C18:1	0.29	0.57	0.62	0.63	0.0811	0.0185	0.1179
trans-10 C18:1	0.61	3.48	4.71	7.02	1.9306	0.0124	0.3347
trans-11 C18:1	2.62	7.18	9.08	7.90	1.9613	0.0273	0.0198
cis-9, *trans*-11 CLA	1.17	1.59	1.24	1.14	0.1731	0.3515	0.0433
trans-10, *cis*-12 CLA	0.13	0.32	0.22	0.33	0.0763	0.0888	0.5175
cis-9, *cis*-12 C18:2 (ω-6)	2.49	2.34	2.24	1.91	0.4168	0.3067	0.8111
cis-9, *cis*-12, *cis*-15 C18:3 (ω-3)	0.29	0.18	0.19	0.14	0.0333	0.0036	0.2191
Ratios between FAs							
C18:0/*trans*-11 C18:1	9.24	4.44	3.43	6.25	1.2830	0.1271	0.0234
C18:0/*trans*-10 C18:1	39.20	20.51	10.89	13.90	7.2149	0.0217	0.3242
trans-11 C18:1/*trans*-10 C18:1	4.27	4.70	3.74	2.18	1.5320	0.0616	0.4970

Table 8
Fatty acid (FA) composition in milk fat from Holstein x Gyr cows fed sugarcane-based diets containing increasing levels of sunflower oil (SO)

Fatty acid (g 100 g^{-1} FA)	SO levels (g kg^{-1} DM)				SEM	P-value	
	0	15	30	45		Linear	Quadratic
Linear even-chain saturated FA							
Σ 4≤C≤10	9.37	8.22	6.98	4.35	0.9097	0.0061	0.4328
C12:0	2.93	2.44	1.63	1.13	0.2844	0.0004	0.9806
C14:0	11.47	10.11	7.85	6.07	0.7700	0.0001	0.6652
C16:0	35.35	29.87	21.69	20.14	2.4593	0.0021	0.4339
Σ 12≤C≤16	49.75	42.42	31.17	27.33	2.8065	0.0006	0.5384
Σ 4≤C≤16	59.11	50.64	38.15	31.67	3.0754	0.0003	0.7367
C18:0	10.06	12.59	14.51	14.48	1.1600	0.0032	0.1229
C18:0 + C20:0 + C22:0 + C24:0	10.31	12.87	14.78	14.77	1.1827	0.0032	0.1244
Odd- and branched-chain FA (OBCFA)							
iso C15:0	0.30	0.21	0.19	0.12	0.0200	<0.0001	0.4107
anteiso C15:0	0.60	0.45	0.52	0.35	0.0310	0.0031	0.7578
C15:0	1.42	1.20	1.09	0.93	0.0565	0.0003	0.5439
C17:0	0.59	0.55	0.56	0.48	0.0392	0.0081	0.3650

continue

continuation

cis-9 C17:1	0.23	0.19	0.15	0.13	0.0180	0.0051	0.6260
Σ OBCFA	3.51	2.88	2.74	2.20	0.1229	0.0003	0.7095
cis-C18:1 FA							
cis-9 C18:1	16.39	18.81	21.05	20.32	1.4597	0.0074	0.0937
cis-11 C18:1	1.11	1.16	1.28	1.36	0.1465	0.1321	0.8839
cis-12 C18:1	0.27	0.52	0.67	0.82	0.0942	0.0052	0.6410
cis-13 C18:1	0.051	0.072	0.235	0.224	0.1011	0.0921	0.8645

Table 9
Fatty acid (FA) composition in milk fat from Holstein x Gyr cows fed sugarcane-based diets containing increasing levels of sunflower oil (SO)

Fatty acid (g 100 g^{-1} FA)	SO levels (g kg^{-1} DM)				SEM	P-value	
	0	15	30	45		Linear	Quadratic
trans-C18:1 FA							
trans-4 C18:1	0.014	0.036	0.041	0.059	0.0058	0.0005	0.6718
trans-5 C18:1	0.017	0.035	0.060	0.081	0.0130	0.0011	0.8896
trans-6-8 C18:1	0.11	0.34	0.44	0.76	0.0815	0.0006	0.5400
trans-9 C18:1	0.18	0.36	0.41	0.75	0.0626	0.0002	0.2617
trans-10 C18:1	0.35	1.17	1.06	3.78	0.8310	0.0076	0.3833
trans-11 C18:1	1.11	2.98	6.26	9.39	1.3960	0.0013	0.5248
trans-12 C18:1	0.18	0.49	0.80	1.38	0.1280	0.0004	0.3341
trans-13-14 C18:1	0.27	0.59	0.78	1.29	0.0791	<0.0001	0.2050
trans-16 C18:1	0.15	0.21	0.60	0.63	0.1521	0.0089	0.9226
Σ *trans*-C18:1	2.37	6.21	10.44	18.12	1.3716	0.0001	0.2015
Conjugated (CLA) and nonconjugated isomers of linoleic acid							
cis-9, *trans*-12 C18:2	0.07	0.14	0.16	0.17	0.0267	0.0107	0.1420
cis-9, *trans*-11 CLA	0.52	1.15	2.41	2.67	0.4643	0.0080	0.6933
trans-9, *cis*-11 CLA	0.034	0.054	0.096	0.108	0.0132	0.0018	0.7250
trans-10, *cis*-12 CLA	0.023	0.053	0.061	0.123	0.0216	0.0015	0.2777
Long-chain polyunsaturated FA							
cis-9, *cis*-12 C18:2 (ω-6)	2.04	2.01	2.70	2.24	0.2123	0.2134	0.3258
cis-6, *cis*-9, *cis*-12 C18:3 (ω-6)	0.101	0.092	0.091	0.058	0.0122	0.0101	0.1744
cis-9, *cis*-12, *cis*-15 C18:3 (ω-3)	0.17	0.13	0.16	0.10	0.0172	0.0536	0.5907
cis-11, *cis*-14 C20:2 (ω-6)	0.025	0.028	0.026	0.024	0.0087	0.8456	0.7118
cis-8, *cis*-11, *cis*-14 C20:3 (ω-6)	0.074	0.056	0.061	0.039	0.0088	0.0269	0.8139
cis-5, *cis*-8, *cis*-11, *cis*-14 C20:4 (ω-6)	0.134	0.086	0.095	0.054	0.0121	0.0054	0.7671

Discussion

The absence of an effect (P>0.05) of SO on DM and NDF_{ap} intake and apparent digestibility (Table 2) corroborates the results of the productive performance trial, presented in our companion paper (Souza et al., 2019). Additionally, the dietary EE content (Table 1) did not exceed 60-70 g kg^{-1} DM (NRC, 2001), and we employed a strategy of mixing the SO with the concentrate (Lopes et al., 2019). This technique probably avoided the deposition of a layer of oil on the forage particles, which could negatively impair both the adhesion of fibrolytic microbiota and the activity of the enzymes involved in cellulose hydrolysis (Jenkins, 1993). Except for a linear reduction (P=0.0254) in the rumen butyrate content, the cows' rumen environments were not

altered by SO and can be considered adequate (pH > 6.0 and N-NH_3 level > 10 mg dL^{-1}; Table 4) for fermentation of fibrous carbohydrates (Valadares & Pina, 2011). Furthermore, the similarity (P>0.05) in degradability parameters for NDF (Table 3) and rumen acetate content (Table 4) demonstrated that the ruminal digestion of the dietary fibrous fractions were not affected by SO.

The diets were isoproteic (Table 1) with similar DM intake (Table 2), and thus, there was no effect of SO on CP intake (Table 2). Additionally, there were no effects on nitrogen metabolism, *i.e*., CP apparent digestibility (Table 2), rumen N-NH_3 content (Table 4), MUN (Table 5), and serum urea nitrogen (Table 6). As expected, the replacement of ground corn and citrus pulp by SO, rich in fat, linearly increased (P<0.0001) the EE intake (Table 2). Relative to the control treatment, EE intake increased by 97%, 184%, and 240% in the diets with 15, 30, and 45 g kg^{-1} SO, respectively. The linear increase in TDN (P=0.0032) in response to SO is a consequence of the replacement of carbohydrate sources (ground corn and citrus pulp) by fat (SO) with a 2.25-fold greater energy content. The EE digestibility also increased with SO (Table 2). Similar results were obtained in the productive performance trial (Souza et al., 2019).

The most consumed FAs were linoleic and oleic acids (Table 2). Relative to the control treatment, SO increased linoleic acid intake by 154%, 301%, and 335%, and oleic acid intake by 101%, 180%, and 185% for the diets with 15, 30, and 45 g kg^{-1} SO, respectively. None of the dietary ingredients are an important source of α-linolenic acid (Souza et al., 2019), and thus, the intake of this FA was low (Table 2). α-Linolenic and linoleic acid, together with oleic acid, are the main substrates for the formation of vaccenic acid in the rumen by partial biohydrogenation (BH) reactions (Shingfield, Bernard, Leroux, & Chilliard, 2010). In the mammary gland, vaccenic acid is the precursor for the synthesis of 70-95% of all rumenic acid secreted in bovine milk (Kliem & Shingfeld, 2016; Prado, Schmidely, Nozière, & Ferlay, 2019). Furthermore, approximately 25% of the vaccenic acid uptake is desaturated into rumenic acid in the mammary gland (Prado et al., 2019).

The absence of an effect (P>0.05) of SO on milk yield (Table 5) can be interpreted as a reflection of the similarities (P>0.05) in DM and TDN intake (Table 2). These data corroborate the results of Souza et al. (2019) in the productive performance trial. The linear increase in milk protein content (P=0.0242) promoted by SO supports the results from some studies (Rodney, Celi, Scott, Breinhild, & Lean, 2015; Lopes et al., 2019), although the literature commonly indicates that lipid supplementation of diets supplied to cows reduces milk protein content (NRC, 2001; Mahdavi, Mahdavi, Darabighane, Mead, & Lee, 2019). The linear reduction in milk fat content was proportionally higher than the linear increase in milk protein content. These changes promoted a linear decrease in milk total solids content because the milk lactose content was not altered by SO (Table 5). Relative to the control treatment, the 45 g kg^{-1} SO diet decreased milk fat content by 29% and slightly increased milk protein content by 6% (Table 5). However, the linear reductions in milk protein (P=0.0242) and total solids (P=0.0046) contents were not sufficient to promote a difference among treatments (P>0.05) in their daily yields (Table 5). Consistent with the absence of a treatment effect on milk yield and lactose content (P>0.05), there was no difference in the daily yield of this component. The absence of an SO-mediated effect on milk lactose content (Table 5) may be a reflection of the similarity (P>0.05) observed among treatments in rumen propionate concentrations (Table 4) and serum glucose content (Table 6).

The negative effect of the dietary inclusion of vegetable oils on cow's milk fat content is well documented in the literature (C. G. S. Ribeiro et al., 2018; Lopes et al., 2019; Mahdavi et al., 2019; Rodrigues et al., 2019). Additionally, the current results corroborate Souza et al. (2019)

in the productive performance trial. The milk fat depression (MFD) observed in the present study can be explained by the linear increase (P<0.05) in the omasal digesta (Table 7) and milk fat (Tables 8 and 9) contents of known FA intermediates of ruminal BH, associated with the inhibition of lipogenesis in the cow mammary gland, *i.e.*, *trans*-10 C18:1, *trans*-10, *cis*-12 CLA, and *trans*-9, *cis*-11 CLA (Shingfield et al., 2010; Bernard et al., 2018; Prado et al., 2019). SO promoted a linear increase in omasal digesta *trans*-10 C18:1 content (P=0.0124) and milk fat *trans*-10 C18:1 (P=0.0076), *trans*-10, *cis*-12 CLA (P=0.0015), and *trans*-9, *cis*-11 CLA (P=0.0018) contents. The regressions (P<0.05) obtained between the milk fat content (y; %) *versus* omasal digesta content (x; g 100^{-1} g FA) of *trans*-10 C18:1 ($\hat{y}$ = -0.08765x + 3.40627) and *trans*-10, *cis*-12 CLA ($\hat{y}$ = -2.01162x + 3.56039) and *versus* the milk fat content (x; g 100^{-1} g FA) of *trans*-10 C18:1 ($\hat{y}$ = -0.16257x + 3.31715), *trans*-10, *cis*-12 CLA ($\hat{y}$ = -6.66674x + 3.49271), and *trans*-9, *cis*-11 CLA ($\hat{y}$ = -8.5327x + 3.67960) demonstrated the effect of these FAs, which are considered to be MFD markers.

Relative to the control treatment, the 45 g kg^{-1} SO diet impressively increased the *trans*-10 C18:1 content in omasal digesta (Table 7) and milk fat (Table 9) 1,051% and 980%, respectively. These data indicate that the *trans*-10 BH pathway of linoleic acid was activated and intensively performed by the ruminal microbiota. The shift from the *trans*-11 to the *trans*-10 alternative BH pathway of linoleic acid is typical of diets supplemented with vegetable oils rich in polyunsaturated FAs (PUFAs). This correlates with changes in the rumen environment, microbial populations, and the amount and type of available FA and carbohydrate substrates (Rico et al., 2015a). Despite the absence of an SO effect on rumen pH (P>0.05), on average, greater than 6.0 for all treatments (Table 4), the pH remained below 6.0 from 6 to 17 h after ration provision (Figure 1). In diets with high PUFA levels, this factor is particularly important because the main microorganisms responsible for the BH processes of these FAs are fibrolytic bacteria that are sensitive to low pH conditions (Rico, Preston, Risser, & Harvatine, 2015b). A decreased ruminal pH alters the microbial population (*e. g*., cellulolytic bacteria) and BH pathways. Consequently, the formation of *trans*-10 18:1 and *trans*-10, *cis*-12 CLA is increased (Fuentes, Calsamiglia, Cardozo, & Vlaeminck, 2009).

The inhibitory effects of *trans*-10, *cis*-12 CLA, *trans*-9, *cis*-11 CLA, and *trans*-10 C18:1 on MFD are associated with the reduction in *de novo* FA synthesis in the mammary gland (Bernard et al., 2018; Prado et al., 2019). The alteration in ruminal fermentation that results in inadequate production of acetate and butyrate to support milk fat synthesis is one theory to explain the mechanism that underlies MFD (Fuentes et al., 2009). In the present study, the rumen acetate content was unchanged by SO, while slight reductions in the rumen butyrate content did not appear to be sufficient to promote the observed MFD. It is likely that unique FAs produced as a result of alterations in the classical linoleic acid BH pathway promoted the inhibition of mammary milk fat synthesis. The high negative correlations (P<0.05) between the milk fat *trans*-10 18:1, *trans*-10, *cis*-12 CLA, and *trans*-9, *cis*-11 CLA contents *versus de novo*-synthesized C4:0 to C16:0 FAs (r = -0.55, -0.70 and -0.73, respectively) indicate that these FAs did in fact inhibit lipogenesis in the mammary gland by reducing *de novo* FA synthesis (Bernard et al., 2018). Therefore, in response to SO, the reduction in the milk fat C4:0 to C16:0 contents (Table 8), which was positively correlated with milk fat content (r = 0.72; P=0.0018), significantly promoted MFD. Another factor that may have contributed to the reduction in the milk fat content of *de novo*-synthesized FAs is the high C18 FA supply to the mammary gland. This phenomenon may modify C18 FA uptake and thus increase competition with short-chain FAs for esterification into the triglycerides of milk fat (Shingfield et al., 2010; Prado et al., 2019). This

hypothesis is supported by negative correlations (r = -0.55 to -0.93, P<0.03) between the sum of the milk fat C4:0 to C16:0 contents *versus* the milk fat contents of several C18-unsaturated FAs (*e.g.*, oleic and rumenic acid, *cis*-9, *trans*-12 C18:2, *trans*-4 C18:1 to *trans*-16 C18:1, *cis*-11 C18:1, *cis*-12 C18:1, *trans*-9, *cis*-11 CLA, and *trans*-10, *cis*-12 CLA). The negative effect of SO on the milk fat content of *de novo*-synthesized FAs has also been observed in the productive performance trial (Souza et al., 2019) and in other studies (C. G. S. Ribeiro et al., 2018; Lopes et al., 2019).

The diets in the present study were isofibrous, with an average of 313 g kg^{-1} NDF_{ap}. However, the SO-supplemented diets presented lower NFC_{ap} and higher EE levels (Table 1) due to the replacement of citrus pulp and ground corn with SO. In response to the lower fermentability of the SO diets, there were linear reductions (P<0.05) in the milk fat content of the major OBCFAs (Table 8). These FAs are found in the lipid membranes of ruminal bacteria and originate from the digestion and absorption of lipids synthesized by the ruminal bacteria, as well as from *de novo* synthesis in the mammary gland (Kliem & Shingfield, 2016). Differences in sensitivity to rumen pH and PUFAs among species and communities of ruminal bacteria may also have contributed to the reduction in milk fat OBCFA content (Rico et al., 2015b). There were consistent negative correlations (P<0.01) between the milk fat OBCFA content and intakes of EE (r = -0.87) and of oleic, linoleic, and α-linolenic acids (r = -0.70 to -0.83). Although the major omasal digesta OBCFA contents were similar (P>0.05) among diets (Table 7), the milk fat contents of odd linear-chain FAs and OBCFAs were dependent on their respective omasal digesta content (r = 0.59, P=0.0159, and r = 0.63, P=0.0096, respectively). This finding indicates that the milk secretion of these FAs was partly ruminal in origin and partly arose from *de novo* mammary synthesis (Prado et al., 2019). Among the OBCFAs, *iso* C15:1 was the only one considered to be a potential MFD marker due to its strong positive association with cows that secreted a typical milk fat content (Conte, Dimauro, Serra, Macciotta, & Mele, 2018). Consistent with Conte et al. (2018), in the present study, among all OBCFAs, *iso* C15:0 was the most proportionally reduced in milk fat (Table 8). Relative to the control treatment and considering the major OBCFAs, the 45 g kg^{-1} SO diet decreased the milk fat *iso* C15:0, *anteiso* C15:0, C15:0, and C17:0 contents by 60%, 42%, 35%, and 19%, respectively.

Corroborating the results obtained in the productive performance trial (Souza et al., 2019), the increased rumen unsaturated FA load, mainly linoleic acid (Table 2), promoted intense ruminal BH. This phenomenon can be verified by the reduction in apparent transfer into milk of ingested linoleic acid (Table 5), the impressive linear increases in the omasal digesta contents of several *trans*-C18:1 FAs (Table 7), and in the milk fat contents of stearic acid (Table 8), *trans*-C18:1 FAs, and conjugated and nonconjugated isomers of linoleic acid (Table 9). Additionally, the absence of an SO effect (P>0.05) on the omasal digesta linoleic acid content (Table 7), despite increases in the intake of this FA in SO diets (Table 2), supports the hypothesis that there was intense BH in the rumen. Due to the high rumen linoleic acid load, the maximum capacity of the *trans*-11 rumen BH pathway was reached at 31.8 g kg^{-1} SO as indicated by the quadratic effect of SO (P=0.0198) on the omasal digesta vaccenic acid content. This phenomenon (Table 7) triggered the need for the microbiota to activate the *trans*-10 alternative ruminal linoleic acid BH pathway. The ratio of *trans*-11 C18:1/*trans*-10 C18:1 FAs in the omasal digesta was numerically decreased (P=0.0616), data that indicate a shift from the *trans*-11 to *trans*-10 ruminal BH pathway of linoleic acid. Relative to the control treatment, the 45 g kg^{-1} SO diet decreased that ratio by 49% (Table 7). The accumulation of *trans*-C18:1 FAs, especially vaccenic acid, *trans*-10 C18:1, and elaidic acid, in the omasal digesta occurred at the expense of stearic acid, whose omasal digesta content was

unchanged by SO (Table 7). This finding partially indicates a reduction in the final step of rumen BH of unsaturated C18 FAs. Reductions (P<0.05) in the omasal digesta ratios of stearic/vaccenic acid and stearic/*trans*-10 C18:1 acid (Table 7) can also be considered indicative of this phenomenon. All changes in omasal digesta FA contents were due to adaptations and changes in the rumen microbiome in response to the unsaturated FA load. However, further investigation is needed to elucidate the functional role of specific bacterial populations in the process of modification of dietary lipids (Rico et al., 2015b).

SO promoted pronounced linear increases (P<0.01) in the milk fat content of all *trans*-C18:1 FAs, including elaidic acid, *trans*-10 C18:1, and vaccenic acid (Table 9). The omasal digesta concentrations of all *trans*-C18:1 FAs were correlated with their appearance in milk (r = 0.51 to 0.68; P<0.05) except for *trans*-10 C18:1, whose omasal digesta content was much higher than its milk fat content. There were marked increases in the milk fat elaidic acid, *trans*-10 C18:1, vaccenic acid, and total *trans*-C18:1 FA contents of 317%, 980%, 746% and 665%, respectively, in the 45 g kg^{-1} SO compared to the control diet (Table 9). These results support those of the productive performance trial (Souza et al., 2019). Elaidic acid and *trans*-10 C18:1 are associated with deleterious effects on cardiovascular health (Vahmani et al., 2017); therefore, an increase in the levels of these FAs in milk fat is not desirable for human nutrition.

Rumenic acid has beneficial effects on human health including decreasing the risk of cancer and type 2 diabetes and improving the immune system (Yang et al., 2015). Diets that promote an increase in the supply of vaccenic acid from the rumen to the mammary gland generally lead to higher rumenic acid levels in milk. This FA is the precursor for the synthesis of 70-95% of rumenic acid in bovine milk (Kliem & Shingfeld, 2016; Prado et al., 2019). This potential is corroborated by the positive relationship ($\hat{y}$ = 0.23468x; r^2_{adj} = 0.79; P<0.0001) between rumenic acid milk fat content (y; g 100 g^{-1} FA) *versus* omasal digesta vaccenic acid content (x; g 100 g^{-1} FA). SO increased the omasal digesta vaccenic acid content (Table 7) as shown by the regressions (P<0.0001) between the omasal digesta content of vaccenic acid (y; g 100 g^{-1} FA) *versus* intakes (x; g cow^{-1} day^{-1}) of oleic ($\hat{y}$ = 0.05008*x; r^2_{adj} = 0.80), linoleic ($\hat{y}$ = 0.02034*x; r^2_{adj} = 0.79) and α-linolenic acids ($\hat{y}$ = 0.41786*x; r^2_{adj} = 0.77). The milk fat rumenic acid contents obtained from 30-45 g kg^{-1} SO diets (Table 9) were higher compared to the range of 0.56-2.05 g 100 g^{-1} FA reported for cows fed diets based on 400-630 g kg^{-1} sugarcane with lipid supplementation (Meneses et al., 2015; Rodrigues et al., 2019). In the productive performance trial, Souza et al. (2019) obtained higher milk fat rumenic acid contents in all SO diets, *i.e.*, 1.46, 2.41, and 2.77 g 100 g^{-1} FA in the diets with 15, 30, and 45 g kg^{-1} SO, respectively. Except for the 45 g kg^{-1} SO diet, higher milk fat vaccenic acid contents were also obtained by Souza et al. (2019), *i.e*., 3.35, 6.40 and 7.56 g 100 g^{-1} FA in diets with 15, 30, and 45 g kg^{-1} SO, respectively. The lower milk rumenic acid contents in the present study can be attributed, at least partly, to the lower milk vaccenic acid contents in association with the lower enzymatic activity of stearoyl-CoA desaturase (SCD) in the rumenic/vaccenic FA pair compared to those presented by Souza et al. (2019). In both studies, SO promoted a linear reduction in SCD activity for the rumenic/vaccenic FA pair.

Some FAs (*e.g.*, *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA) were associated with a decrease in SCD-catalyzed FA desaturation in the bovine mammary gland (Kadegowda, Bionaz, Piperova, Erdman, & Loor, 2009). However, in the present study, none of the correlations between the milk fat content of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA *versus* all the SCD indices were significant (P>0.05). On the other hand, there were negative correlations (P<0.05) between SCD activity for rumenic/vaccenic *versus* specific FAs (stearic acid, *cis*-13 C18:1, *trans*-16 C18:1, C20:0, and C24:0). Stearic

acid is a substrate that competes with vaccenic acid for SCD in oleic acid synthesis. Stearic acid showed a high negative correlation with SCD activity for the rumenic acid/vaccenic acid pair (r = -0.79; P=0.0003). A linear increase (P=0.0074) in the milk fat oleic acid content was observed in SO treatments (Table 8).

The absence of an SO effect on serum glucose content (Table 6) corroborates the results obtained in the productive performance trial (Souza et al., 2019) and those presented by Rodrigues et al. (2017, 2019). No SO effect on serum NEFA contents was observed, data that are consistent with the results presented by Rodrigues et al. (2019). The obtained values (Table 6) were within the normal range for healthy cows, <0.4 mmol L^{-1} (J. E. P. Santos, 2011). The increase in serum cholesterol and triglycerides would be expected due to increased FA transport in the blood in response to supplementation with vegetable oils (Rodrigues et al., 2017, 2019; Souza et al., 2019). For serum cholesterol content, this increase was observed (P<0.05). Despite the absence of a treatment effect (P>0.05) on serum triglycerides, the SO diets promoted numerically higher values compared to the control (Table 6).

Corroborating the results obtained in the productive performance trial (Souza et al., 2019), SO improved the indices of nutritional quality for milk fat: AI, TI, and the h/H FA ratio (data not shown). There were linear decreases (P<0.01) in milk fat hypercholesterolemic FAs (lauric, myristic and palmitic acids) and a linear increase (P=0.0074) in milk fat oleic acid (Table 8), a FA associated with beneficial effects on cardiovascular health (Hammad, Pu, & Jones, 2016).

Conclusions

Up to 45 g kg^{-1} sunflower oil can be included on dry matter of chopped sugarcane-based diets without reducing consumption, apparent digestibility and rumen degradability of dry matter and fiber.

Supplementing chopped sugarcane-based diets with 30 to 45 g kg^{-1} sunflower oil (dry matter basis) promotes milk fat depression due to the inhibition of mammary lipogenesis by specific rumen-derived fatty acid intermediates in the biohydrogenation of unsaturated C18 fatty acids.

The inclusion of 15 to 45 g kg^{-1} sunflower oil in chopped sugarcane-based diets improves the nutritional quality of milk fat, with increases in the levels of oleic, vaccenic and rumenic acids, which are beneficial to human health, and reductions in the levels of hypercholesterolemic lauric, myristic and palmitic acids.

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