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Butter fatty acid composition as a function of soybean oil supplementation and time of milking, and performance of Holstein x Gyr cows fed with chopped elephant grass-based diets

Perfil de ácidos graxos na manteiga em função da suplementação com óleo de soja e do horário da ordenha e desempenho de vacas Holandês x Gir alimentadas com dietas à base de capim-elefante picado

Fernando César Ferraz Lopes[1*]; Carlos Gustavo Santos Ribeiro[2]; Norberto Mario Rodriguez[3]; Marco Antônio Sundfeld da Gama[4]; Mirton José Frota Morenz[4]; Rosemar Antoniassi[5]; Humberto Ribeiro Bizzo[5]

Abstract

The aim of this study was to evaluate the performance of cows fed with a total mixed ration composed of elephant grass and a concentrate containing 0.0% (control), 1.5%, 3.0% or 4.5% soybean oil on a dry matter basis. The effect of milking time (morning *versus* afternoon) on butter fatty acid composition was also evaluated. Twelve multiparous Holstein x Gyr cows with an average milk production of 18.0±4.6 kg day^{-1} and 90±25 days in milk were used in a triplicated 4 x 4 Latin square experimental design with 15-day periods. Data were analyzed using mixed models. There was no effect of soybean oil supplementation on dry matter or neutral detergent fiber intake ($P > 0.05$). The fat-corrected milk yield linearly decreased ($P=0.0109$) and the milk protein yield linearly increased ($P=0.0023$) in response to soybean oil supplementation. The butterfat *trans*-9 C18:1, *cis*-9 C18:1, *trans*-10 C18:1, *trans*-11 C18:1, *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA content linearly increased ($P < 0.05$), whereas the C12:0, C14:0 and C16:0 content linearly decreased ($P < 0.001$) as the level of soybean oil in the diet increased. The butter produced from afternoon milk had a lower content of C16:0 and a higher content of *cis*-9 C18:1 ($P < 0.05$). The supplementation of elephant grass-based-diets with soybean oil and, to a small extent, the selective segregation of milk obtained from afternoon milking sessions are strategies that can be used to improve the fatty acid composition of butterfat.

Key words: Conjugated linoleic acid. Milk composition. *Pennisetum purpureum*.

[1] Analista, Embrapa Gado de Leite, EMBRAPA, Juiz de Fora, MG, Brasil. E-mail: fernando.lopes@embrapa.br
[2] Prof., Instituto Federal de Educação, Ciência e Tecnologia de Brasília, IFB, Campus Planaltina, Brasília, DF, Brasil. E-mail: carlos.ribeiro@ifb.edu.br
[3] Prof., Departamento de Zootecnia, Escola de Veterinária, Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, MG, Brasil. E-mail: norberto.bhe@terra.com.br
[4] Pesquisadores, Embrapa Gado de Leite, Juiz de Fora, MG, Brasil. E-mails: marco.gama@embrapa.br; mirton.morenz@embrapa.br
[5] Pesquisadores, Embrapa Agroindústria de Alimentos, EMBRAPA, Rio de Janeiro, RJ, Brasil. E-mail: rosemar.antoniassi@embrapa.br; humberto.bizzo@embrapa.br
[*] Author for correspondence

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Resumo

O objetivo deste estudo foi avaliar o desempenho de vacas alimentadas com capim-elefante picado e concentrado com inclusão de 0,0% (controle), 1,5%, 3,0% e 4,5% de óleo de soja na matéria seca da dieta. Além da suplementação com óleo de soja, também foi avaliado o efeito do horário de ordenha (manhã *versus* tarde) sobre o perfil de ácidos graxos na manteiga. Foram utilizadas 12 vacas Holandês x Gir com 90±25 dias em lactação, produzindo 18.0±4.6 kg dia^{-1} de leite, e alocadas em delineamento com três Quadrados Latinos 4 x 4. Os resultados foram analisados por modelos mistos. A suplementação com óleo de soja não alterou os consumos de matéria seca e de fibra em detergente neutro ($P > 0,05$). A suplementação com óleo de soja promoveu redução linear ($P=0,0109$) na produção de leite corrigida para gordura e aumento linear ($P=0,0023$) na produção de proteína. Foi observado aumento linear ($P < 0,05$) nos teores dos ácidos graxos C18:1 *trans*-9, C18:1 *cis*-9, C18:1 *trans*-10, C18:1 *trans*-11, CLA *cis*-9, *trans*-11 e CLA *trans*-10, *cis*-12 na manteiga, e redução linear ($P < 0,001$) nos teores dos ácidos graxos C12:0, C14:0 e C16:0 em resposta à suplementação com óleo de soja. A manteiga produzida do leite da ordenha da tarde apresentou menor teor de C16:0 e maior teor de C18:1 *cis*-9 ($P < 0,05$). A suplementação de dietas à base de capim-elefante com óleo de soja e, em menor escala, a segregação seletiva de leite obtido na ordenha da tarde são estratégias que podem ser utilizadas para melhorar a composição de ácidos graxos da manteiga.

Palavras-chave: Ácido linoleico conjugado. Composição do leite. *Pennisetum purpureum*.

Introduction

In Brazil, elephant grass (*Pennisetum purpureum*, Schumach) is one of the most important tropical grasses to grow in a cutting-and-carry system for feeding dairy cattle (PEREIRA et al., 2016). Well-managed elephant grass used for cutting contains high levels of linoleic (*cis*-9, *cis*-12 C18:2) and α-linolenic (*cis*-9, *cis*-12, *cis*-15 C18:3) polyunsaturated-fatty acids (LOPES et al., 2015). These fatty acids (FA) are the main substrates for the formation of vaccenic acid (*trans*-11 C18:1) in the rumen. In the mammary gland, vaccenic acid is the precursor for the synthesis of 70% to 95% of all rumenic acid (*cis*-9, *trans*-11 CLA), the major isomer of CLA (conjugated linoleic acid) secreted in bovine milk (KLIEM; SHINGFIELD, 2016). Rumenic acid is a biologically active FA and a potential health-promoting factor. Anticarcinogenic, antidiabetogenic (type 2 diabetes), antiatherogenic and immunomodulatory properties have been attributed to this CLA isomer (YANG et al., 2015). Because the main source of rumenic acid in the human diet is ruminant milk fat (YANG et al., 2015), the production of dairy products enriched with this FA and other health-enhancing FAs has been the subject of research carried out in Brazil (LOPES et al., 2015) and worldwide (KLIEM; SHINGFIELD, 2016).

The inclusion of vegetable oils in the ration of cows fed with chopped elephant grass improved the nutritional quality of milk fat, with an increase in the content of oleic (*cis*-9 C18:1), rumenic and vaccenic FAs, which are beneficial to human health, and a concomitant reduction in hypercholesterolemic saturated FAs such as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids (RIBEIRO et al., 2014, 2018).

On the other hand, rations formulated with high dietary levels of vegetable oils rich in polyunsaturated FAs may cause an increase in the formation of FA intermediates of ruminal biohydrogenation (BH), such as *trans*-10, *cis*-12 CLA and *trans*-9, *cis*-11 CLA (RIBEIRO et al., 2018). These FAs (and possibly others) are associated with the inhibition of lipogenesis in the mammary gland (SHINGFIELD et al., 2010; JENKINS; HARVATINE, 2014; BERNARD et al., 2018), promoting milk fat depression (MFD).

Numerous factors are involved in the modulation of the vaccenic and rumenic acid contents in milk fat. Of these, the most important factors are those related to diet composition and the nutritional management of cows (*e.g.*, forage species/cultivars, pasture or chopped grass *versus* typical feed rations, lipidic supplementation, supply of forage in the form of silage, hay or zero-grazing feeding system, etc.). However, factors such as cow breed, lactation stage, lactation order, season of the year, forage/pasture agronomic management (*e.g.*, fertilization, regrowth stage, irrigation, etc.), climate, milk production system (*e.g.*, organic *versus* conventional), in addition to the individual variation itself, are also important aspects in obtaining milk naturally enriched with vaccenic and rumenic acids (ELGERSMA, 2015; LOPES et al., 2015).

Another factor not yet studied in Brazil that has the potential to produce milk naturally enriched with FAs that are beneficial to human health, according to studies carried out in temperate regions, is the selective segregation of milk obtained during P.M. milking (FERLAY et al., 2010; SCHWENDEL et al., 2015; VIBART et al., 2017). To illustrate this, in the studies of Ferlay et al. (2010), Rico et al. (2014) and Schwendel et al. (2015), lower levels of the hypercholesterolemic saturated FAs lauric, myristic and palmitic acid were obtained in milk that was acquired in the P.M. In addition, these authors also reported higher levels of FAs that are beneficial to human health (*e.g.*, oleic, vaccenic and rumenic acids) in milk that was obtained in the P.M. Based on the results of these and other studies, it was hypothesized that in the present study, the butter produced from milk that was milked in the afternoon would have an FA composition with a higher nutritional quality. The proof of this hypothesis may provide the rural producer and the dairy industry with an additional strategy that can be used to improve the FA composition of butterfat.

The present study provides results of dairy cow performance that complement those presented in the companion paper of Ribeiro et al. (2018), which studied the association between ruminal metabolism and the milk FA composition in cows fed chopped elephant grass-based diets supplemented with the same dietary levels of soybean oil. However, instead of FA composition in milk, this study presents results of the FA composition in butter produced from milk obtained during morning and afternoon milking session, a study unheard of in Brazil.

The aim of this study was to evaluate the effect of soybean oil supplementation and time of milking (morning *versus* afternoon) on the butter fatty acid composition and the performance of Holstein x Gyr dairy cows fed chopped elephant grass-based diets.

Materials and Methods

The study was carried out at Embrapa Dairy Cattle (Coronel Pacheco, Minas Gerais, Brazil). Twelve multiparous (3-4 lactations) dairy cows (503±48 kg) with genetic composition varying from 3/4 to 15/16 Holstein x Gyr at the initial third of lactation (90±25 days) and producing 18.0±4.6 kg day^{-1} of milk at the beginning of the experiment were used. All of the experimental procedures with animals were carried out according to Embrapa Dairy Cattle guidelines for animal care and use in research.

The experimental design was a triplicated 4 x 4 Latin square (LS) in which each period consisted of 10 days for diet adaptation and five days for sampling and data collection. The cows were homogeneously distributed in each LS based on the genetic composition in crossbreeding, daily milk yield, days in milk, body weight and lactation order.

The experimental treatments were rations based on chopped elephant grass with inclusion (diet DM basis) of 0.0% (control), 1.5%, 3.0% or 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}. The cows were collectively

allocated in a free-stall and were provided with fresh water and a mineral mixture. The free-stall was equipped with electronic troughs (American Calan Inc., Northewood, NH, USA) which allowed individual control of food consumption. The diets were provided to the cows as a total mixed ration (TMR) once a day (08h00) using a mixer/dispenser vehicle (Data Ranger; American Calan Inc., Northewood, NH, USA). The roughage:concentrate ratio was of 46:54 (DM basis), and the rations were supplied after the morning milking session in amounts to allow for 10% orts. The Napier cultivar of elephant grass was used, and it was cut every two days and chopped daily. The concentrates with SO were prepared weekly to avoid lipid peroxidation.

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, % DM				
Chopped elephant grass[a]	46.0	46.0	46.0	46.0
Ground corn	17.0	16.3	15.1	14.4
Soybean meal	19.0	19.0	19.8	19.6
Citrus pulp	17.0	16.3	15.1	14.4
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	44.0	42.9	44.4	45.5
Crude protein (CP)	15.1	13.5	15.9	14.3
Ether extract (EE)	1.63	3.74	4.54	6.18
Non-fibrous carbohydrates	30.0	28.7	27.1	25.7
Neutral detergent fiber (NDF)	45.9	47.1	45.1	46.7
Acid detergent fiber (ADF)	31.9	32.3	29.2	31.1
Lignin	7.4	6.5	6.4	6.6
Mineral matter	8.9	8.7	8.3	8.3
Fatty acid composition, % DM				
Palmitic acid	0.537	0.790	0.870	1.161
Stearic acid	0.081	0.149	0.184	0.259
Oleic acid	0.371	0.647	0.880	1.096
Linoleic acid	0.701	1.335	1.951	2.478
α-Linolenic acid	0.094	0.166	0.245	0.309
RUFAL[d]	1.2	2.1	3.1	3.9

[a]25.7% DM, 5.8% CP, 1.2% EE, 73.8% NDF, 11.0% lignin and 42.0% DM *in vitro* digestibility; [b]Veleiro® (Cargill Agrícola S.A., Uberlândia, MG, Brazil); [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; [d]*Rumen Unsaturated Fatty Acid Load* (total amount of dietary unprotected unsaturated-FA entering the rumen on a daily basis and their potential to trigger milk fat depression) = Σ dietary oleic + linoleic + α-linolenic acids (MANNAI et al., 2016).

From the 11th to the 15th 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. The samples of the chopped elephant grass and the four concentrates were collected daily and stored (-10°C) for chemical analyses. The samples of elephant grass and the four concentrates were transformed into composites per LS period and stored (-10°C). The 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, predried (55°C, 72 h), milled (1 mm) and analyzed for DM (at 105°C), crude protein (CP), ether extract (EE), mineral matter (MM) and neutral detergent fiber (NDF), according to Detmann et al. (2012). For the analysis of the FA composition, performed in the Laboratory of Fats and Oils of Embrapa Food Technology (Rio de Janeiro, RJ), the samples of chopped elephant grass were transformed into composites per week (n = 8) and were analyzed together with the samples of the four experimental concentrates collected on the first and seventh day after their preparation. The frozen samples of these foods were lyophilized, ground and subjected to digestion according to the method 996.06 (AOAC…, 2005), and the methylation was performed according to Hartman and Lago (1973). The FA methyl esters were analyzed using gas chromatography (model Agilent 6890N, Agilent Technologies, Palo Alto, CA, USA) with a fused silica capillary column of cyanopropyl siloxane (60 m x 0.32 mm x 0.25 μm) and a flame ionization detector.

The cows were mechanically milked twice a day (06h00 and 14h00) with milk yields recorded from the 11th to the 15th 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 FA profile study was performed on butter samples, produced from the pool of milk that was milked from the groups of three cows from each experimental treatment, obtained in the A.M. (morning) and P.M. (afternoon) milking on the 11th day of each LS period. Thus, 32 experimental butters (four treatments x two milking x four LS periods) were produced at Embrapa Dairy Cattle (Coronel Pacheco, MG, Brazil), according to the process described by Gonzalez et al. (2003). The butters that were obtained were stored (-80°C), and at the end of the experimental period, they were analyzed at the Laboratory of Chromatography of Embrapa Dairy Cattle (Juiz de Fora, MG, Brazil) for the FA composition according to Ribeiro et al. (2018).

The nutritional quality of butterfat 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 equations described by Ribeiro et al. (2018).

Indices of Stearoyl-CoA Delta-9 desaturase (SCD) activity were calculated according to Gama et al. (2008), as the product/precursor ratio.

The results were analyzed by mixed models using the MIXED procedure of SAS version 9.1 (SAS..., 2004). The butter FA composition, and the indices of nutritional quality of butterfat and of the activity of SCD were analyzed as repeated measures over time (time of milking), and were considered to be fixed effects: treatment (level of SO), time of milking (A.M. *versus* P.M.) and the interaction between these factors; and random effects: group, LS period, and the interactions of time of milking with group and with LS period. The variable "group" corresponds to the butter produced from the pool of milks milked from three cows receiving the same experimental diet in each LS period. Ten matrices of covariance were evaluated, based on the Akaike information criterion. The variables concerning to performance of the cows (nutrients intake, milk yield and composition, and feed efficiency) were analyzed considering treatment as a fixed effect; and LS, cow within LS and LS period as random effects. The linear and quadratic effects were analyzed using orthogonal contrasts. The results were reported as LSM. Significant differences were declared at $P \leq 0.05$. Regression equations between specific variables were adjusted by the REG procedure and *Pearson* correlations obtained by the CORR procedure of SAS.

Results and Discussion

There was no effect (P>0.05) of SO supplementation on DM and NDF intake (Table 2). To avoid a reduction in DM intake, especially in the initial phase of lactation, NRC (2001) recommends formulating rations with a maximum of 6-7% EE (DM basis). However, special care should be taken when using vegetable oil, which has a rapid release in the rumen and a potential deleterious effect on the resident microbiota (JENKINS; HARVATINE, 2014). According to Jenkins (1993), the deposition of the oil layer on the fodder particles can impair both the adhesion of the fibrolytic microbiota and the activity of the enzymes involved in the cellulose hydrolysis, causing a negative impact on the digestion of the fibrous fraction of the diet, consequently, on the DM intake. Thus, it is likely that the SO inclusion in the concentrate, which was supplied to cows in a TMR with elephant grass, contributed to avoiding a possible negative impact of SO on DM intake (NRC, 2001), especially in the ration with 4.5% SO, whose EE content was 6.2% (Table 1). These results corroborate those reported by Ribeiro et al. (2014, 2018) which also provided chopped elephant grass mixed with concentrates containing added vegetable oils, and no difference was observed in DM intake by lactating cows.

As expected, in response to the replacement of the ingredients (ground corn, soybean meal) of the concentrates by SO (Table 1), there was a linear increase (P<0.0001) in EE intake (Table 2). Consequently, there were linear reductions in non-fibrous carbohydrates – NFC (P=0.0405) and MM (P=0.0133) intake. Regarding the control treatment, there was a 237% increase in EE intake and a 6.4% reduction in NFC intake of cows fed a 4.5% SO diet. The quadratic effect (P<0.0001) on CP intake (Table 2) was observed, with a minimum value of 1.4 kg cow^{-1} day^{-1} estimated when 3.1% SO was included in the diet. Due to similar DM intake among treatments, CP intakes were a reflection of the differences in rations of the CP contents (Table 1).

Milk yield was not altered by SO supplementation (P>0.05). The similar DM intake among treatments may justify this response (Table 2), corroborating the results of Ribeiro et al. (2018). However, the SO supplementation promoted a negative linear effect (P<0.0001) on milk fat and lactose content. The negative effect of the dietary inclusion of vegetable oils on the milk fat content of cows is well documented in the literature (LOPES et al., 2015; RODRIGUES et al., 2017; RIBEIRO et al., 2018). Milk fat in the 4.5% SO ration was reduced 16% compared to the control ration. Rations formulated with high dietary levels of vegetable oils rich in polyunsaturated-FAs may cause an increase in the formation of FA intermediates of ruminal BH, such as *trans*-10, *cis*-12 CLA and *trans*-9, *cis*-11 CLA (RIBEIRO et al., 2018). These FAs and possibly others, such as *trans*-10 C18:1, are associated with the inhibition of lipogenesis in the mammary gland (SHINGFIELD et al., 2010; JENKINS; HARVATINE, 2014; BERNARD et al., 2018), promoting MFD. The mechanisms responsible for the inhibitory effects on milk fat synthesis triggered by *trans*-10, *cis*-12 CLA or possibly by any other rumen-derived FA (*e.g.*, *trans*-9, *cis*-11 CLA and *trans*-10 C18:1) are not clearly elucidated. In cows, the MFD occurs in concert with a dramatic decrease of the expression of the main genes involved in *de novo* FA synthesis, long chain FA uptake, or triacylglycerol synthesis, as well as their regulatory elements (transcription factors) (BERNARD et al., 2018). The linear increments (P<0.001) observed in the butterfat contents of rumen-derived bioactive FAs *trans*-10, *cis*-12 CLA, *trans*-10 C18:1 and *trans*-9, *cis*-11 CLA in response to SO supplementation (Table 3) justify the MFD observed in the present study, particularly in rations with 3.0% and 4.5% SO (Table 2).

Table 2. Daily nutrient intake and milk yield and composition of Holstein x Gyr dairy cows fed chopped elephant grass-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
Nutrient intake (kg day^{-1})							
Dry matter (DM)	16.9	17.0	16.8	17.0	1.1168	0.9452	0.8376
Neutral detergent fiber	9.30	9.48	9.12	9.29	0.6311	0.6821	0.9721
Crude protein	1.87	1.37	1.49	1.43	0.0950	<0.0001	<0.0001
Ether extract	0.38	0.72	0.87	1.28	0.0506	<0.0001	0.2117
Mineral matter	1.60	1.71	1.53	1.53	0.1057	0.0133	0.1060
Non-fibrous carbohydrates	3.76	3.77	3.72	3.52	0.2629	0.0405	0.2175
Fatty acid intake (g day^{-1})							
C16:0	90.8	134.6	145.8	197.8	8.9054	<0.0001	0.2523
C18:0	13.7	25.5	30.8	44.1	1.7737	<0.0001	0.3633
cis-9 C18:1	62.7	110.3	147.3	186.8	7.8946	<0.0001	0.2553
cis-9, *cis*-12 C18:2	118.6	227.6	326.3	422.3	17.0647	<0.0001	0.4177
cis-9, *cis*-12, *cis*-15 C18:3	16.0	28.3	40.9	52.6	2.1487	<0.0001	0.7407
Nutrient intake (kg 100 kg^{-1} of body weight)							
Dry matter	3.42	3.49	3.45	3.51	0.1461	0.4445	0.9223
Neutral detergent fiber	1.88	1.94	1.88	1.91	0.0811	0.7880	0.7149
Yield (kg cow^{-1} day^{-1})							
Milk	17.8	18.7	18.2	18.1	1.4117	0.7614	0.1110
FCM[a]	16.1	16.3	15.4	15.0	1.5877	0.0109	0.4340
Fat	0.601	0.586	0.542	0.521	0.0702	0.0004	0.8422
Protein	0.577	0.610	0.599	0.622	0.0522	0.0023	0.5196
Lactose	0.805	0.832	0.812	0.792	0.0672	0.3931	0.1493
Total solids	2.142	2.056	1.948	1.818	0.2851	0.0894	0.9872
Milk composition (%)							
Fat	3.37	3.11	2.94	2.84	0.1867	<0.0001	0.2288
Protein	3.23	3.25	3.30	3.46	0.1246	0.0001	0.0852
Lactose	4.52	4.44	4.45	4.37	0.0474	<0.0001	0.9329
Total solids	12.02	10.71	10.65	9.96	0.9343	0.1475	0.6495
Feed efficiency							
kg of milk/kg of DM intake	1.06	1.10	1.09	1.05	0.0428	0.7306	0.1330
kg of FCM/kg of DM intake	0.96	0.95	0.91	0.88	0.0573	0.0053	0.4693

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

Table 3. Effect of time of milking (A.M. *versus* P.M.) and levels of soybean oil (SO) on butter fatty acid (FA) composition*.

FA	SO levels (% of diet DM)					P-value		Time of milking			
(g 100 g^{-1} FA)	0.0	1.5	3.0	4.5	SEM	L	Q	AM	PM	SEM	P-value
Linear even-chain saturated FAs											
Σ 4≤C≤10	9.37	8.26	7.13	6.11	0.425	<0.001	0.785	7.70	7.73	0.424	0.896
C4:0	3.56	3.29	3.09	2.74	0.179	<0.001	0.684	3.10	3.23	0.165	0.210
C12:0	3.05	2.48	1.97	1.67	0.130	<0.001	0.083	2.33	2.26	0.123	0.472
C14:0	11.15	9.50	7.98	6.97	0.410	<0.001	0.186	9.10	8.70	0.374	0.104
C16:0	29.65	24.44	22.50	20.64	0.654	<0.001	0.028	25.15	23.45	0.601	0.018
C18:0	7.63	9.17	9.25	9.79	0.245	<0.001	0.029	9.05	8.87	0.233	0.388

continue

continuation

C20:0	0.12	0.13	0.13	0.14	0.003	0.002	0.193	0.13	0.13	0.003	0.658
C22:0	0.054	0.058	0.058	0.045	0.004	0.097	0.029	0.055	0.053	0.004	0.756
C24:0	0.010	0.007	0.005	0.003	0.001	<0.001	0.732	0.006	0.007	0.001	0.526
Σ 12≤C≤16	43.85	36.42	32.44	29.27	1.070	<0.001	0.049	36.58	34.41	1.043	0.033
Σ 4≤C≤16	53.22	44.67	39.57	35.39	1.430	<0.001	0.142	44.28	42.14	1.340	0.078
Σ C ≥ 18	7.81	9.37	9.45	9.97	0.249	<0.001	0.028	9.24	9.06	0.236	0.387
Odd- and branched-chain FAs – OBCFA											
C11:0	0.31	0.25	0.19	0.14	0.012	<0.001	0.436	0.21	0.23	0.013	0.148
C13:0	0.095	0.071	0.059	0.050	0.004	<0.001	0.061	0.069	0.069	0.003	0.967
iso C15:0	0.34	0.29	0.24	0.19	0.010	<0.001	0.578	0.25	0.28	0.009	0.076
anteiso C15:0	0.60	0.51	0.44	0.37	0.013	<0.001	0.675	0.46	0.50	0.009	0.008
C15:0	1.12	0.97	0.85	0.75	0.024	<0.001	0.624	0.92	0.93	0.022	0.632
iso C16:0	0.26	0.22	0.19	0.17	0.009	<0.001	0.256	0.20	0.22	0.008	0.082
iso C17:0	0.66	0.60	0.57	0.52	0.009	<0.001	0.535	0.58	0.59	0.009	0.407
anteiso C17:0	0.84	0.73	0.64	0.57	0.023	<0.001	0.212	0.68	0.71	0.020	0.024
C17:0	0.60	0.55	0.47	0.45	0.023	<0.001	0.541	0.51	0.53	0.013	0.133
cis-9 C17:1	0.27	0.22	0.21	0.18	0.014	<0.001	0.452	0.20	0.24	0.012	0.003
C21:0	0.007	0.012	0.018	0.018	0.002	<0.001	0.204	0.014	0.014	0.001	0.911
C23:0	0.131	0.105	0.083	0.059	0.006	<0.001	0.834	0.082	0.107	0.005	0.006
Σ a*nteiso*-FA	1.43	1.24	1.09	0.94	0.033	<0.001	0.363	1.14	1.21	0.027	0.012
Σ *iso*-FA	1.26	1.11	1.00	0.88	0.016	<0.001	0.230	1.04	1.09	0.018	0.039
Σ OLCFA	2.53	2.18	1.89	1.64	0.055	<0.001	0.336	2.01	2.12	0.040	0.013
Σ OBCFA	5.23	4.52	3.98	3.46	0.097	<0.001	0.244	4.18	4.41	0.079	0.010
cis-monounsaturated FAs											
cis-9 C12:1	0.068	0.055	0.051	0.053	0.005	0.031	0.146	0.061	0.053	0.004	0.085
cis-9 C14:1	1.37	1.10	0.97	0.82	0.066	<0.001	0.305	1.02	1.11	0.053	0.136
cis-9 C16:1	1.72	1.32	1.27	1.19	0.078	<0.001	0.012	1.32	1.43	0.066	0.067
cis-9 C18:1	19.94	21.40	22.55	22.27	0.865	0.017	0.168	20.61	22.47	0.732	<0.001
cis-11 C18:1	0.57	0.64	0.69	0.77	0.028	<0.001	0.805	0.67	0.67	0.027	0.685
cis-12 C18:1	0.27	0.52	0.65	0.81	0.030	<0.001	0.138	0.57	0.55	0.029	0.641
cis-13 C18:1	0.073	0.083	0.092	0.096	0.003	<0.001	0.173	0.086	0.086	0.003	0.908
cis-11 C20:1	0.059	0.059	0.065	0.081	0.005	0.001	0.070	0.061	0.071	0.004	0.031
trans-C18:1 FAs											
trans-4	0.020	0.050	0.054	0.056	0.004	<0.001	0.009	0.043	0.047	0.004	0.392
trans-5	0.018	0.041	0.048	0.055	0.002	<0.001	0.002	0.042	0.039	0.002	0.242
trans 6-8	0.23	0.53	0.66	0.77	0.047	<0.001	0.077	0.56	0.53	0.046	0.591
trans-9	0.33	0.50	0.58	0.60	0.030	<0.001	0.005	0.51	0.50	0.036	0.956
trans-10	0.48	1.22	1.37	2.56	0.238	<0.001	0.865	1.45	1.37	0.226	0.867
trans-11	1.90	4.51	6.34	8.30	0.543	<0.001	0.516	5.49	5.03	0.413	0.369
trans-12	0.35	0.57	0.70	0.79	0.031	<0.001	0.048	0.61	0.59	0.030	0.606
trans 13-14	0.48	0.81	0.92	1.15	0.048	<0.001	0.260	0.89	0.80	0.049	0.135
trans-16	0.36	0.52	0.61	0.65	0.016	<0.001	0.001	0.54	0.53	0.016	0.913
Σ *trans*-C18:1	4.17	8.75	11.29	14.94	0.595	<0.001	0.437	10.12	9.45	0.584	0.307
Nonconjugated linoleic acid isomer											
trans-9, *cis*-12	0.21	0.21	0.18	0.17	0.013	0.017	0.563	0.18	0.20	0.013	0.233
Conjugated linoleic acid (CLA) isomers											
cis-9, *trans*-11	1.23	2.57	3.67	4.50	0.233	<0.001	0.202	2.91	3.08	0.191	0.384
trans-9, *cis*-11	0.019	0.037	0.053	0.072	0.005	<0.001	0.936	0.045	0.046	0.003	0.561
trans-10, *cis*-12	0.001	0.010	0.017	0.029	0.003	<0.001	0.687	0.016	0.013	0.002	0.412

continue

continuation

Long-chain polyunsaturated FAs											
LA (ω-6)	2.24	2.33	2.38	2.36	0.080	0.145	0.326	2.25	2.41	0.073	0.101
GLA (ω-6)	0.044	0.035	0.029	0.029	0.005	0.020	0.261	0.029	0.039	0.005	0.062
LNA (ω-3)	0.27	0.28	0.25	0.22	0.018	0.048	0.346	0.27	0.24	0.018	0.169
EA (ω-6)	0.032	0.033	0.036	0.030	0.005	0.895	0.492	0.037	0.029	0.004	0.077
DGLA (ω-6)	0.090	0.069	0.070	0.059	0.005	<0.001	0.278	0.064	0.079	0.005	0.014
ARA (ω-6)	0.029	0.023	0.020	0.017	0.001	<0.001	0.144	0.022	0.023	0.001	0.399
EPA (ω-3)	0.040	0.034	0.031	0.027	0.002	<0.001	0.771	0.033	0.033	0.001	0.944
Σ *cis* ω-3	0.31	0.31	0.28	0.25	0.018	0.019	0.356	0.30	0.28	0.018	0.177
Σ *cis* ω-6	2.44	2.49	2.53	2.49	0.082	0.448	0.407	2.40	2.58	0.078	0.115
Ratios between FAs											
C18/*t*-11 C18:1	4.08	2.14	1.53	1.24	0.150	<0.001	0.001	2.22	2.27	0.146	0.686
C18/*t*-10 C18:1	16.86	7.89	7.13	4.67	1.131	<0.001	0.079	9.70	8.57	1.031	0.347
C18/*t*-19 C18:1	22.86	18.58	16.27	16.65	0.808	<0.001	0.003	18.83	18.34	0.785	0.633
t-11/*t*-10 C18:1	4.19	3.70	4.75	4.38	0.891	0.904	0.602	4.61	3.89	0.787	0.170
Spreadability	1.513	1.154	1.001	0.935	0.073	<0.001	0.026	1.242	1.060	0.062	0.001

*The interaction levels of SO*time of milking was not significant (P>0.05) for any FA.
Abbreviations: L = linear effect; Q = quadratic effect; SEM = standard error of the mean; Σ 4≤C≤10 = C4:0 + C6:0 + C8:0 + C10:0; Σ 12≤C≤16 = C12:0 + C14:0 + C16:0; Σ C ≥ 18 = C18:0 + C20:0 + C22:0 + C24:0; Σ *trans*-C18:1 FAs = *trans*-4 C18:1 + *trans*-5 C18:1 + *trans* 6-8 C18:1 + *trans*-9 C18:1 + *trans*-10 C18:1 + *trans*-11 C18:1 + *trans*-12 C18:1 + *trans*-13-14 C18:1 + *trans*-16 C18:1; OLCFA (odd-linear-chain FA) = C11:0 + C13:0 + C15:0 + C17:0 + *cis*-9 C17:1 + C21:0 + C23:0; Σ *anteiso*-FA = *anteiso* C15:0 + *anteiso* C17:0; Σ *iso*-FA = *iso* C15:0 + *iso* C16:0 + *iso* C17:0; Σ OBCFA = Σ *anteiso*-FA + Σ *iso*-FA + Σ OLCFA; LA = linoleic acid (*cis*-9, *cis*-12 C18:2); GLA = γ-linolenic acid (*cis*-6, *cis*-9, *cis*-12 C18:3); LNA = α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3); EA = eicosadienoic acid (*cis*-11, *cis*-14 C20:2); DGLA = dihomo-γ-linoleic acid (*cis*-8, *cis*-11, *cis*-14 C20:3); ARA = araquidonic acid (*cis*-5, *cis*-8, *cis*-11, *cis*-14 C20:4); EPA = eicosapentaenoic acid (*cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 C20:5); Σ *cis* ω-3 = LNA + EPA; Σ *cis* ω-6 = LA + GLA + EA + DGLA + ARA; Spreadability index (MARTIN et al., 2005) = C16:0/*cis*-9 C18:1.

A shortage in the availability of stearic acid for endogenous oleic acid synthesis by SCD combined with an increase in the supply of rumen-derived *trans*-C18:1 FAs at the mammary gland have been proposed as another possible mechanism to explain part of the decrease in milk fat secretion during diet-induced MFD (SHINGFIELD et al., 2010; BERNARD et al., 2018). In the chopped elephant grass-based diets supplemented with sunflower oil (LOPES et al., 2015) or SO (RIBEIRO et al., 2018), the reported levels of stearic acid in milk (11.1-16.6 g 100 g^{-1} FA) were always higher than those presented in Table 3. This indicates that in the present study there was some restriction in the supply of stearic acid for desaturation in the mammary gland. Thus, because *trans*-C18:1 isomers have a higher melting point than the equivalent *cis*-C18:1 isomers, the replacement from oleic acid to *trans*-C18:1 in the *sn*-3 position of triacylglycerol would be expected to decrease milk fat fluidity (GAMA et al., 2008). This increase in the melting point of milk fat would exceed the ability of the mammary gland to regulate milk fluidity and to ensure its efficient ejection and, consequently, decrease the milk fat secretion (BERNARD et al., 2018).

The linear reduction (P<0.0001) in milk lactose content may be associated with the linear reduction (P=0.0405) in NFC intake in diets with SO (Table 2). The milk total solids content were not affected by SO supplementation, since the reductions in milk fat and lactose contents were compensated by a linear increase (P=0.0001) in milk protein content (Table 2), which did not present a correlation (P>0.05) with CP intake. In general, the literature indicates that the lipid supplementation of diets supplied to cows promotes a reduction in milk protein content (NRC, 2001). However, this issue needs more studies, considering that recent meta-analysis studies have

demonstrated that the supplementation of the diets with oilseed promoted both reduction (RABIEE et al., 2012) and increment (RODNEY et al., 2015) in the bovine milk protein content.

Despite the similarity (P>0.05) in the milk yield among treatments, significant effects (P<0.001) on fat and milk protein contents promoted linear reduction (P=0.0004) and linear increase (P=0.0023), respectively, in their daily yield (Table 2). Inversely, the linear reduction (P<0.0001) in milk lactose content was not enough to promote a difference (P>0.05) in its daily yield among treatments. Corroborating the absence of the effect of treatments on milk yield and total solids content (P>0.05), no difference (P=0.0894) was observed in the daily yield of this component. There was no effect (P>0.05) of treatments on the feed efficiency expressed as kg of milk/kg of DM intake (Table 2). This result is consistent with the absence (P>0.05) of the effects of treatments on milk yield and on DM intake. However, due to the lower fat yields in the rations with SO (P=0.0004), a linear reduction (P=0.0053) was observed for the feed efficiency based on the 4% fat-corrected milk yield.

Except for the α-linolenic acid content, which was generally lower than the mean values reported for the chopped elephant grass (22.0-55.0 g 100 g^{-1} FA), the other majority of FAs were present in levels (Table 4) that were within of the ranges reported in the literature (LOPES et al., 2015). In the four experimental concentrates, collected on the first and the seventh day after their preparation, in general, changes in the contents of the majority of FAs were minimal (Table 4). These results indicate that within one week after the preparation, the concentrates, particularly those with SO, remain suitable for use in feeding cows, with no impairment in their composition due to the lipid peroxidation of unsaturated FAs. Because the linoleic acid was the major FA in the experimental concentrates (Table 4) and rations (Table 1), it was the main FA consumed by the cows (Table 2). In relation to the control treatment, the inclusion of SO promoted an expressive increase in its intake of 92%, 175% and 256%, respectively, in the rations with 1.5%, 3.0% and 4.5% SO. The second unsaturated-FA that was most consumed by cows was oleic acid (Table 2), which was present in high levels in SO concentrates (Table 4) and rations (Table 1). The α-linolenic acid, whose main dietary source was the chopped elephant grass (Table 4), was the third unsaturated-FA that was most consumed by cows (Table 2). It should be noted that these three unsaturated-C18 FAs are the substrates for the formation of vaccenic acid in the rumen, by partial BH reactions (SHINGFIELD et al., 2010).

Table 4. Fatty acid (FA) composition (g 100 g^{-1} FA) of chopped elephant grass and concentrates.

Fatty acid	Chopped elephant grass[b]	Soybean oil levels (% of diet DM)[a]							
		0.0%		1.5%		3.0%		4.5%	
		Day after preparation of the concentrate							
		1st day	7th day	1st day	7th day	1st day	7th day	1st day	7th day
Palmitic	35.41 ± 4.44	19.19	19.10	15.96	15.40	14.17	14.26	13.77	14.36
Stearic	4.38 ± 0.66	3.37	3.33	3.41	3.46	3.36	3.41	3.32	3.43
Oleic	8.86 ± 3.06	24.64	24.36	24.15	23.53	23.10	23.28	22.66	23.06
Linoleic	20.76 ± 3.41	45.56	45.74	48.50	49.28	51.51	51.11	51.82	51.43
Linolenic	17.75 ± 3.66	3.83	3.93	4.69	4.94	5.61	5.48	5.80	5.61

[a]Soybean oil (Veleiro®; Cargill Agrícola S.A., Uberlândia, MG, Brazil) = 11.17; 3.27; 24.01; 53.78 and 6.50 g 100 g^{-1} FA, respectively for palmitic, stearic, oleic, linoleic and α-linolenic acids; [b]Mean ± standard deviation (*n* = 8 samples).

However, under specific conditions that promote disturbance in ruminal fermentation, a shift from the *trans*-11 to the *trans*-10 pathway of polyunsaturated-FAs BH, may lead to the production of excessive amounts of *trans*-10, *cis*-12 CLA (MANNAI et al., 2016), as observed in the present study (Table 3). The *trans*-10, *cis*-12 CLA produced in the rumen travel via the blood to the mammary gland, where it inhibits the synthesis of milk fat by impairing the expression and production of several lipogenic enzymes essential for milk fat synthesis (JENKINS; HARVATINE, 2014). According to these authors, a useful tool to monitor the risk of unsaturated-FA intake to trigger MFD is called "Rumen Unsaturated Fatty Acid Load" (RUFAL). RUFAL reflects the total unsaturated-FA supply entering the rumen each day from feed. In the present study, the rations with 3.0% and 4.5% SO presented RUFAL of 3.1 and 3.8, respectively (Table 1). RUFAL values larger than 3.5% DM were considered by Jenkins and Harvatine (2014) to present a risk of provoking MFD. In fact, the milks from the treatments with 3.0% and 4.5% SO had fat contents below the minimum of 3.0% stipulated by Normative Instruction 62 (BRASIL, 2011), indicating the occurrence of MFD.

The interaction levels of SO*time of milking were not significant (P>0.05) for any FA (Table 3) or indices (Table 5). Therefore, the discussion of the effects of these factors will be performed independently.

The linear increase (P<0.0001) in the unsaturated-C18 FA intake (Table 2) in response to SO supplementation promoted an intense ruminal BH. The FA composition of the butterfat confirms this (Table 3), since there was a generalized increases (P<0.05) in the contents of known intermediates of ruminal BH of oleic, linoleic and α-linolenic acids (*e.g., trans*-6-16 C18:1, *cis*-11-12 C18:1, *trans*-9, *cis*-12 C18:2, rumenic acid, *trans*-9, *cis*-11 CLA and *trans*-10, *cis*-12 CLA). Following digestion and absorption, these rumen-derived FAs can be incorporated into the milk fat (SHINGFIELD et al., 2010). Furthermore, even with the significant increases (P<0.0001) in the consumption of linoleic and α-linolenic acids in response to SO supplementation (Table 2), there was no increase in their contents in butterfat (Table 3), also indicating that both were extensively biohydrogenated in the rumen.

It was a quadratic effect (P=0.029) on stearic acid content in butterfat (Table 3), with a maximum value of 9.7 g 100 g^{-1} FA, estimated when 4.2% SO was included in the DM of the ration. This result is indicative of a reduction in the extent of the ruminal BH of unsaturated-C18 FAs (Table 3). Stearic acid is the common end product of the main ruminal BH pathways and, in general, is the major FA leaving the rumen (SHINGFIELD et al., 2010). However, the increase in the butterfat stearic acid content was only 28% in the 4.5% SO ration compared to the control ration, indicating a reduction in the final step of BH in response to SO supplementation. This can be further verified by the linear (P<0.001) reductions in the C18:0/*trans*-11 C18:1 and C18:0/*trans*-10 C18:1 ratios (Table 3). According to McKain et al. (2010), the bacterium *Butyrivibrio proteoclasticus* performs the final step of BH, converting vaccenic and *trans*-10 C18:1 FAs into stearic acid in the rumen. Therefore, a possible reduction in its population in the rumen due to the toxic effects of polyunsaturated-FAs could promote the accumulation of *trans*-C18:1 FAs at the expense of stearic acid.

Table 5. Effect of time of milking (A.M. *versus* P.M.) and levels of soybean oil (SO) on indices of nutritional quality of butterfat and on indices of the activity of Stearoyl-CoA desaturase enzyme.*

Index	SO levels (% of diet DM)							Time of milking			
	0.0	1.5	3.0	4.5	SEM[b]	Linear	Quad	AM	PM	SEM[b]	P-value
Nutritional quality of butterfat[a]											
AI	3.47	2.71	2.23	2.03	0.195	<0.001	0.098	2.79	2.43	0.164	0.018
TI	4.01	3.34	2.95	2.85	0.185	<0.001	0.072	3.50	3.08	0.153	0.021
h/H FA	0.47	0.60	0.71	0.77	0.041	<0.001	0.028	0.59	0.68	0.039	0.013
ω-6/ω-3 FA	7.95	8.28	9.02	10.03	0.303	<0.001	0.234	8.22	9.42	0.220	0.011
Activity of SCD enzyme (product/precursor ratio)											
c-9 C12:1/C12:0	0.023	0.022	0.026	0.032	0.003	0.027	0.270	0.027	0.025	0.002	0.091
c-9 C14:1/C14:0	0.123	0.116	0.122	0.118	0.008	0.633	0.692	0.112	0.127	0.007	0.003
c-9 C16:1/C16:0	0.058	0.054	0.056	0.058	0.003	0.872	0.073	0.052	0.061	0.003	<0.001
c-9 C17:1/C17:0	0.449	0.408	0.448	0.397	0.022	0.190	0.841	0.405	0.446	0.023	0.110
c-9 C18:1/C18:0	2.614	2.350	2.442	2.282	0.080	0.003	0.390	2.301	2.543	0.068	<0.001
R/V[c]	0.655	0.583	0.587	0.554	0.030	0.001	0.261	0.556	0.633	0.029	<0.001

*The interaction levels of SO*time of milking was not significant (P>0.05) for any index.
[a]Calculated according to Ribeiro et al. (2018): AI = atherogenicity index; TI = thrombogenicity index; h/H FA = hypo/hypercholesterolemic fatty acids ratio; ω-6/ω-3 FA = omega 6/omega 3 fatty acids ratio; [b]Standard error of the mean; [c]R/V = rumenic acid/vaccenic acid ratio.

Ruminal BH is a detoxification mechanism performed by cellulolytic bacteria to prevent the bacteriostatic effects of polyunsaturated-FAs (MAIA et al., 2010). The main pathway of ruminal BH of linoleic acid is carried out by *Butyrivibrio fibrisolvens* bacteria, with the formation of rumenic and vaccenic acids (*trans*-11 BH pathway) (McKAIN et al., 2010). In the present study, the importance of this BH pathway can be attested by the linear increase (P<0.001) in the butterfat contents of rumenic and vaccenic acids (Table 3). The increases in rumenic and vaccenic acid were 266% and 337%, respectively, in the butterfat contents from cows fed the 4.5% SO ration compared to the control ration. However, the high input of linoleic acid in the rumen can trigger the need to adapt the ruminal microbiota for the execution of BH by alternatives pathways (RICO et al., 2015; PITTA et al., 2018). The impressive increases in the butterfat contents of *trans*-10, *cis*-12 CLA and *trans*-10 C18:1 (Table 3) of 2,800% and 433% (P<0.001), respectively, in the 4.5% SO ration compared with the control ration indicate that the *trans*-10 BH pathway of linoleic acid was activated and concomitantly carried out by the ruminal microbiota. The absence of effect (P>0.05) on the *trans*-11 C18:1/*trans*-10 C18:1 ratio (Table 3) shows that on the day the milks were collected, there was no prevalence of one BH pathway over the other.

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 and is preceded by a remarkable reduction on the relative abundance of bacterial populations (species richness) and their distribution (diversity) in the rumen (RICO et al., 2015; PITTA et al., 2018). SO supplementation promoted generalized linear decreases (P<0.001) in butterfat contents of odd- and branched-chain FAs (OBCFA) (Table 3). This can be considered as 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. Moreover, the substitution of NFC from 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.

The butterfat contents of vaccenic and rumenic acid obtained in the milk of cows treated with 4.5% SO (Table 3) were quite impressive. These contents were higher than the respective ranges of 3.11 to 7.97 g 100 g^{-1} FA and 1.22 to 3.24 g 100 g^{-1} FA compiled by Lopes et al. (2015) in studies in which cows were fed with chopped elephant grass-based diets supplemented with 1.3% to 4.5% sunflower oil. The rumenic acid content in the butter obtained with 4.5% SO is also higher than all values compiled by Shingfield et al. (2013) in the milk of cows submitted to different types of nutritional management; this further demonstrates the magnitude of the butterfat rumenic acid content obtained in the present study. In the companion study carried out by Ribeiro et al. (2018), high milk contents of rumenic and vaccenic acids were also obtained. However, in the study of Ribeiro et al. (2018), although the vaccenic acid content in the milk of cows fed the 4.5% SO ration was higher than that obtained in the present study (9.76 *versus* 8.30 g 100 g^{-1} FA), this result was not reflected in the milk rumenic acid content, which, although quite high, was lower than that presented in Table 3 (3.73 *versus* 4.50 g 100 g^{-1} FA). The regressions of butterfat contents of rumenic *versus* vaccenic acid (g 100 g^{-1} FA) obtained in the present study ($\hat{y}$ = 0.47720*x + 0.48298; r^2 = 0.93; P<0.0001) and in the companion study carried out by Ribeiro et al. (2018) ($\hat{y}$ = 0.33603*x + 0.4027; r^2 = 0.88; P<0.0001) help to explain these results. The value of the regression coefficient obtained in the present study was numerically higher (0.47720 *versus* 0.33603), indicating a higher conversion rate from vaccenic acid to rumenic acid. A possible explanation for the higher value in the present study concerns the use of Co-EDTA as a liquid phase marker by Ribeiro et al. (2018). This marker was associated with a decrease in SCD-catalyzed desaturation of FAs in the bovine mammary gland (SHINGFIELD et al., 2008; LESKINEN et al., 2016).

SO supplementation promoted a generalized reduction in the butterfat contents of *de novo*-synthesized FAs (C4:0 to C16:0). The reduction in the milk contents of *de novo*-synthesized FAs in response to the supplementation of the chopped elephant grass-based diets with the inclusion of vegetable oils is well documented (RIBEIRO et al., 2014; LOPES et al., 2015; RIBEIRO et al., 2018). The reductions (P<0.001) in the butterfat contents of lauric, myristic and palmitic acids were 45%, 38% and 30%, respectively, in the 4.5% SO ration compared to the control ration (P<0.001). As such, due to their characteristic high contents in lauric, myristic and palmitic FAs, the consumption of whole milk, butter and cheese have been noted for their possible adverse effects on the biomarkers of cardiovascular risk (*e.g.*, serum LDL-cholesterol levels). However, recent studies have shown the absence of a relationship between the consumption of saturated FAs from dairy products and the risk of cardiovascular disease (BERNARD et al., 2018).

The high negative correlation between the butterfat contents of *trans*-10, *cis*-12 CLA *versus* the sum of concentrations of *de novo*-synthesized FAs C4:0 to C16:0 (r = -0.75; P<0.0001) can be considered indicative that this CLA isomer inhibited, at least in part, the lipogenesis in the mammary gland via the reduction of *de novo* FA synthesis (BERNARD et al., 2018). Negative correlations (P<0.05) were also observed between *de novo*-synthesized FAs *versus* the rumen-derived bioactive FAs *trans*-9, *cis*-11 CLA and *trans*-10 C18:1. Another hypothesis to explain the reduction in the butterfat contents of *de novo*-synthesized FAs (Table 3) concerns the preferential incorporation of mono- and polyunsaturated-FAs into the triglycerides of the milk fat (SHINGFIELD et al., 2010). This hypothesis is supported by the negative correlations (r = -0.46 to -0.95, P<0.01) between the sum of the butterfat contents of *de novo*-synthesized FAs *versus* those of several C18-unsaturated FAs (*e.g.*, oleic, rumenic, linoleic, *trans*-4-16 C18:1, *cis*-11-13 C18:1, *trans*-9, *cis*-11 CLA).

SO supplementation promoted a linear increase (P=0.017) in the butterfat content of oleic acid (Table 3). This effect is desirable for human health due to the association of oleic acid with reducing the risk of cardiovascular disease. However, this increase in oleic acid was only 12% in the 4.5% SO ration compared to the control ration, probably due to the linear reduction (P=0.003) in SCD activity in response to SO supplementation (Table 5). *Trans*-10, *cis*-12 CLA may have inhibited the activity of the SCD enzyme (KADEGOWDA et al., 2009), since negative correlations (P<0.01) were observed between the butterfat content of this rumen-derived bioactive FA *versus* the SCD activity on the stearic/oleic pair (r = -0.51%).

The major *trans*-monounsaturated isomers intermediates of the ruminal unsaturated-C18 FAs BH pathways are elaidic (*trans*-9 C18:1), *trans*-10 C18:1 and vaccenic acid (SHINGFIELD et al., 2010); the increases in the butterfat contents of these three FAs were 82%, 433% and 337% in the 4.5% SO ration compared to the control ration (Table 3). In a study realized *in vitro* with human liver cells (HepG2), Vahmani et al. (2017) reported that *trans*-6-, *trans*-9- and *trans*-10-C18:1 induced lipogenic/cholesterolgenic gene expression resulting in increased (P<0.05) cellular content of triacylglycerol (TG) and cholesteryl esters (CE). Conversely, *trans*-11 C18:1 and other isomers (*i.e.*, *trans*-13-, *trans*-14-, and *trans*-15-C18:1) resulted in similar amounts of TG and CE compared to *cis*-9 C18:1 treated cells. These results illustrate that increasing elaidic and *trans*-10 C18:1 contents in dairy products is not desirable for human health. However, while SO supplementation promoted a linear increase (P<0.001) in the butterfat contents of elaidic and *trans*-10 C18:1 acids (Table 3), these FAs are below or in the range of values compiled by Kliem and Shingfield (2016) in studies with vegetable oils in the diets supplied to cows.

The SO supplementation improved the nutritional quality of butter, since there was a linear reduction (P<0.001) in AI and TI, and a linear increase (P<0.001) in the h/H FA ratio (Table 5). This can be attributed mainly to the decrease in the butterfat contents of the hypercholesterolemic lauric, myristic and palmitic acids; the concomitant increase in oleic acid content; and specifically considering TI, the increase in the butterfat content of stearic acid (Table 3). The increase (P<0.001) in the ω-6/ω-3 FA ratio in response to SO supplementation (Table 5) was a consequence of the linear reduction (P=0.0019) in the butterfat ω-3 FAs content (α-linolenic and EPA), since there was no effect (P>0.05) of the treatments on the butterfat ω-6 FAs content (Table 3).

The butter produced from milk from the cows that were milked in the afternoon presented FA composition with a higher nutritional quality, since there was a reduction (P<0.05) of ~12-13% in AI and TI and an increase (P=0.013) of 15% in the h/H FA ratio (Table 5). These results were mainly due to differences in the butterfat contents of oleic and palmitic acids, since minor changes were observed in the butterfat FA composition as a function of milking time (Table 3). The butter produced from milk from the cows that were milked in the afternoon showed a lower content of palmitic acid (P=0.0018) and higher contents (P<0.05) of oleic acid, *cis*-11 C20:1, dihomo-γ-linoleic acid (*cis*-8, *cis*-11, *cis*-14 C20:3) and of specific OBCFA (*anteiso* C15:0, *anteiso* C17:0, *cis*-9 C17:1 and C23:0). The other FAs presented similar contents (P>0.05) in the butters produced from milks that were milked in the morning or afternoon. There was no effect (P>0.05) of the milking time on the butterfat contents of ω-6 and ω-3 FAs (Table 3), but due to small numerical differences, the butter produced from milk from the cows that were milked in the afternoon presented a higher (P=0.011) ω-6/ω-3 FA ratio (Table 5).

Differences in the milk FA composition as a function of milking time have been associated with either the time that feeds were offered (or grazed) or the intervals between the milking (FERLAY et al., 2010; SCHWENDEL et al., 2015; VIBART et al., 2017). For cows housed in free-stalls and fed once daily with TMR, the highest peaks of daily

food consumption occur within 4 h after dietary supply (MÄNTYSAARI et al., 2016). Thus, under the conditions of the present study, the higher availability and, consequently, BH of unsaturated-FA in the rumen of cows could be expected in the period between the time the diet was supplied (morning) and the afternoon milking session. However, in the butter produced from afternoon milk, there was no increase (P>0.05) in the content of vaccenic acid (the main *trans*-C18:1 isomer in bovine milk) or in that of any rumen-derived *trans*-C18:1 FAs (Table 3). Thus, the absence of the effect of milking time on the butterfat content of vaccenic acid was reflected in a similar content of rumenic acid, although the activity of the SCD enzyme was 14% higher (P<0.001) in the butter produced from milk from the cows that were milked in the afternoon (Table 5). In contrast to the present study, higher concentrations of rumenic and vaccenic acids were obtained in milk from the cows that were milked during the P.M. milking session (FERLAY et al., 2010; RICO et al., 2014; SCHWENDEL et al., 2015; VIBART et al., 2017).

The highest concentration of specific OBCFAs and the 5-6% increments (P<0.05) in the sums of *iso*-, *anteiso*- and odd-linear chain FAs in the butter produced from milk from the cows that were milked in the afternoon (Table 3) can be explained, at least in part, by the supposed increase in microbial synthesis in the rumen (FERLAY et al., 2010). Between the time the diet was supplied in the morning and the afternoon milking session, it can be assumed, based on the results indicated in the companion study (RIBEIRO et al., 2018), that there was an adequate ruminal environment (pH and ammonia nitrogen content) for the activity of the populations of fibrolytic bacteria.

These results corroborate the literature regarding the higher concentration of oleic acid and the lower palmitic acid in milk from cows milked during the P.M. milking (FERLAY et al., 2010; RICO et al., 2014; SCHWENDEL et al., 2015). Some of these results can be explained by the higher (P<0.001) SCD enzyme activity for the pairs oleic/stearic and palmitoleic/palmitic acids (Table 5). In the companion study (RIBEIRO et al., 2018), peak ruminal acetate content occurred 12.2 h postprandial, that is, after the afternoon milking session. In this sense, Ferlay et al. (2010) reported that the higher palmitic acid content in the milk from the cows that were milked in the morning might be linked to the supply of precursors (acetate and β-hydroxybutyrate) of *de novo*-FAs synthesis in the mammary gland.

Hardness and spreadability are inversely related aspects of the texture of the butters that greatly influence consumer acceptability (WRIGHT et al., 2001). Butter notoriously possesses poor spreadability at refrigeration temperatures, and studies aiming to improve this rheological property of butter have been accomplished (LIN et al., 1996; SHÄFFER et al., 2001). According to Couvreur et al. (2006), modifications in FA composition, and in particular, the decrease in the spreadability index (C16:0/C18:1 ratio) were responsible for linear decreases in the final butter melting temperature, perceived in a sensory analysis by a linear decrease in firmness in the mouth. In the present study, the butter spreadability, as estimated by the palmitic/oleic ratio (MARTIN et al., 2005), linearly decreased (P<0.001) in response to SO supplementation (Table 3). The observed decrease in the 4.5% SO ration compared to the control ration was 38%. In addition to this, afternoon milk produced butter that was 17% more (P=0.001) spreadable (Table 5). These results are related to the higher concentration of oleic acid and to the lower concentration of palmitic acid in butter produced from milk from cows that were milked in the afternoon, as well as in the butters obtained from the diets that were SO-supplemented. It should be noted that the oleic acid and palmitic acid have a low and high melting point, respectively, and were the main unsaturated-FA and saturated-FA in the butters (Table 3).

Conclusions

The inclusion of up to 4.5% soybean oil in elephant grass-based diets reduces fat-corrected milk yield and increases milk protein yield in Holstein x Gyr cows. The supplementation of elephant grass-based diets with soybean oil and, to a small extent, the selective segregation of milk obtained from afternoon milking sessions are strategies that can be used to improve the fatty acid composition of butterfat.

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