

Original Research Paper

# Fatty Acid Profile in Butter from Cows Fed with Sunflower Seeds with or Without Lignosulfonate

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**Abstract:** The objective of this study was to evaluate the fatty acid composition of butter from Holstein cows fed diets containing ground sunflower seeds or ground and pelleted sunflower seeds, with or without the addition of lignosulfonate. The butter was produced from milk collected from eight Holstein cows multiparous, with  $100 \pm 62.6$  days in milk, with a mean weight of  $556 \pm 39.8$  kg Body Weight (BW) and producing  $15.6 \pm 0.12$  kg of Milk/day. The cows were distributed in a double Latin square, with 4 periods of 21 days each, with 14 days for adaptation and seven days for data collection. Diets containing pelleted sunflower seeds altered the fatty acids profile of the butter, with an increase in the fatty acids 16:1n-11, 18:1trans and CLA18:2 c9 t11. However, decreased the amounts of fatty acids 18:0 and 20:0. The amounts of Saturated Fatty Acids (SFA) decreased, while the Polyunsaturated Fatty Acids (PUFA) and the amount of total Conjugated Linoleic Acid (CLA) increased with the pelleted diet. Thus, the use of diets with pelleted sunflower seeds is possible to obtain butter with desirable nutritional value in dairy products, which are beneficial for consumer health.

**Keywords:** Conjugated Linoleic Acid, Dairy Products, Pellet, Polyunsaturated Fatty Acids

## Introduction

Several foods with natural components rich in nutrient pharmaceutical properties are studied to prevent diseases (Tanaka, 2005), present only in vegetables. However, Conjugated Linoleic Acid (CLA) is the exception because it is exclusively present in ruminant products. The CLA represents a group of isomers of linoleic acid, which is found in derivatives of ruminants. The CLA 18:2c9t11 is a featured isomer with interesting nutritional properties and improves human health by reducing body fat and preventing hyperglycemia (Barendse, 2014). According to Hernández *et al.* (2007), fatty acid profiles demonstrates that CLA is a stable component in the dairy products and concluded that dietary supplementation of sunflower seed in cows increases the CLA contents in butter, which may

contribute to the reduction of the risk of cardiovascular diseases in humans.

When formulating concentrates for ruminants, is important increase the energy density of the diet (Mir *et al.*, 2003). Lipid supplements are frequently included in ruminant diets for increase the energy density of the diet while simultaneously increasing the nutrient utilization (Mangrum *et al.*, 2016).

The sunflower seeds are rich in Polyunsaturated Fatty Acids (PUFA), mainly linoleic fatty acid, with 47% oil in the composition. According to Figueiroa *et al.* (2013) with 11% of Saturated Fatty Acids (SFA), 27% Monounsaturated (MUFA) and 60% Polyunsaturated (PUFA), 60.5% of which are linoleic acid. The addition of either sunflower whole seeds or oil to lactating goats ration had beneficial effects on milk yield and milk composition with enhancing milk content of healthy

fatty acids (CLA and omega 3), without detrimental effects on animal performance (Morsy *et al.*, 2015).

Biohydrogenation occurs in rumen, by ruminal microorganisms, against the toxicity of Unsaturated Fatty Acids (UFAs) (Valente *et al.*, 2016). The PUFA such as C18:2 have a diene double bond configuration, in the rumen environment occurs the first step in the biohydrogenation process. Is an isomerization reaction that converts the cis-12 double bond of UFAs into a trans-11 isomer. The last step of the biohydrogenation process is the reduction of vaccenic acid to stearic acid (C18:0). The extent to which trans-11 C18:1 is hydrogenated to C18:0 depends on the conditions that are prevalent in the rumen. Complete hydrogenation to stearic acid is promoted by the presence of ruminal fluid and feed particles and is inhibited irreversibly by the presence of large amounts of linoleic acid (Berg *et al.*, 2006).

There are some ways of protecting dietary ingredients by avoiding contact with ruminal microorganisms, reducing ruminal biohydrogenation. Among the methods, pelleting can improve the nutrient utilization efficiency of feeds, which also improvement the production of milk and dairy products (Neves *et al.*, 2007). The heat treatment may protect partially the biohydrogenation of linoleic acid. Studies on the feeding of dairy cows with the supply of soybean and canola grains thermally treated confirm the increase in the concentration of isomer 18:2 c9 t11 (Neves *et al.*, 2009). The lignosulfonate is a by-product from the wood industry, enzymatic agglomerate rich in xylose and can be added to the ruminant diet with the aim of reducing ruminal biohydrogenation and support the pelletizing process (Figueiroa *et al.*, 2013). The addition of lignosulfonate to the diet of dairy cows had no negative effect on dry matter intake, milk production, milk concentrations of Crude Protein (CP), lactose and total solids (Santos *et al.*, 2011). Among the properties of lignosulfonate its potential to be combined with proteins. Petit *et al.* (1999) utilized fat soybeans and lignosulfonate and observed that lignosulfonate reduced the ruminal degradation of proteins, thus increasing the levels ruminal non-degradable proteins by protecting them from the activity of rumen microorganisms and a decrease in Dry Matter (DM) digestibility upon increased temperature and the addition of lignosulfonate. The established hypotheses are: It is possible to improve the fatty acid profile of milk and dairy products by supplying sunflower seeds to dairy cows; Physical treatments and chemical properties of sunflower seeds may reduce the appearance of saturated fatty acids in butter. The objective of this study was to evaluate the fatty acid profile in milk butter from Holstein cows fed diets containing sunflower seeds or pelleted sunflower

seeds, with or without lignosulfonate addition, under grazing conditions.

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## Materials and Methods

### Experimental Site

The study was carried out in Experimental Farm of Iguatemi (FEI), at the State University of Maringá, Brazil. Located at 23°21'19.2"S, 52°04'22.8"W, average altitude of 551 m. The climate of the region is classified as Cfa by the Köppen classification (humid subtropical climate).

### Animal Management, Feeding and Treatment

A group of eight Holstein cows multiparous, with 100±62.6 days of milk, with a mean weight of 556±39.8 kg body weight (BW) and producing 15.6±0.12 kg of Milk/day. The cows were distributed in a double Latin square, with 4 periods of 21 days each, with 14 days for adaptation to diet and seven days for data collection.

The diets consisted of four concentrates, with the sunflower seed (only ground or ground and pelleted) and the addition or not of lignosulfonate, in a 2×2 factorial arrangement.

The experimental diets were: 1- Ground sunflower seeds (S); 2- Ground sunflower seeds with addition of lignosulfonate (SL); 3- Ground sunflower seeds and pelleted (SP); 4- Ground Sunflower seeds with addition of Lignosulfonate and Pelleted (SLP). Nutritional composition of the diets is shown in Table 1.

Sunflower seeds used in the four concentrates were ground through a 5 mm screen using a Nogueira® DPM-2 chopper and added at 427.4 g/kg of dry matter (DM). The lignosulfonate solution was prepared with Lignosol (Melbar®) and had 740 g/kg and contained 27 g/kg DM of xylose. The lignosulfonate solution was added at 50 g/kg DM after grinding the seeds. Pelletizing of concentrates for treatments was with a 40 HP pelleting machine (Chavantes®) without steam addition at a 75°C exit temperature. Yield averaged 900 kg/h and the die diameter was 4.5 mm. New batches of concentrates were made for each of the four periods but the same lot of ground sunflower seeds was used for the whole experiment.

**Table 1:** Percentage composition of the diets on a dry matter (DM) basis

Ingredients (%)	Treatment			
	S	SL	SP	SLP
Soybean meal	23.14	23.14	23.14	23.14
Cracked corn	22.53	22.53	22.53	22.53
Dicalcium phosphate	4.70	4.70	4.70	4.70
Mineral mix and vitamin <sup>a</sup>	3.10	3.10	3.10	3.10
Sodium bicarbonate	1.55	1.55	1.55	1.55
Limestone	1.40	1.40	1.40	1.40
NaCl	0.84	0.84	0.84	0.84
Ground sunflower seeds	42.74	-	-	-
Ground sunflower seeds with addition of lignosulfonate	-	42.74	-	-
Ground sunflower seeds and pelleted	-	-	42.74	-
Ground sunflower seeds with addition of lignosulfonate and pelleted	-	-	-	42.74

<sup>a</sup>Ca 270 g/kg, P 80 g/kg, S 20 g/kg, Mg, 15 g/kg, Fe 2200 mg/kg, Cu 800 mg/kg, Co 50 mg/kg, I 60 mg/kg, Se: 40 mg/kg, Zn 2800 mg/kg, F 801 mg/kg, Vit. A 216000 U.I./kg, Vit. D 67600 U.I./kg, Vit. E 500 mg/kg

Cows were kept in pasture of (*Cynodon dactylon* (L.) Pers.) cultivars Coast Cross (CC) and Bermuda Grass (BG). Four periods were used for the study. Grazing was initiated when pasture height equaled or exceeded 30 cm and terminated at a height of 20 cm. Cows were individually fed experimental concentrates for a 45 min period after each milking. Intake of concentrates was recorded for each cow after each feeding and amounts offered were adjusted to allow 100 g/kg refusals as fed. Milk production was recorded at every milking.

Pasture samples were obtained on day 15 and 21 after throwing a metallic square with dimension (1.0 m<sup>2</sup>) three times within the most recently grazed paddock (n = 12 samples/intake period). Samples of pasture were pooled on a period basis by paddock to obtain one sample/period for chemical analysis. Pastures were grazed to a 20 cm stubble height. Composite samples of concentrates, refusals and pasture were mixed thoroughly and sub-sampled for chemical analysis.

The Forage: Concentrate ratio was 70:30. The diets were formulated to be isoproteic and isoenergetic, based on ethereal extract, according to NRC (2001). The cows received concentrate feed individually in a *Tie stall* barn, in two meals per day at 8 and 16 h, immediately after morning and afternoon milking, remaining for about 45 min at a time.

To maintain the Forage: Concentrate ratio of feed intake, it was necessary to calculate fecal output, a capsule of chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) was inserted into the rumen of the fistulated cows (n = 4) and given orally to the non-fistulated cows (n = 4) once daily at 09:00 h from day 11 to 20, supplying a total of 15 g of Cr<sub>2</sub>O<sub>3</sub>/d. Samples of feces (100 g) were collected from all cows for 6 consecutive days at 08:00, 10:00, 12:00, 14:00, 16:00 and 18:00 h of each experimental period, respectively, for days 15-20 and kept frozen (-20°C). Fecal samples were dried at 55°C for 48 h and ground to pass a 1 mm screen in a Wiley mill. Equal DM from each fecal sub-sample was mixed to obtain a single

composite for each sampled cow during each experimental period. After thawing, samples of feed and forage were weighed and oven dried at 60°C for 72 h. Then, samples were processed in a Wiley® knife mill to pass through 1-mm screen sieves and stored in plastic bags. Samples were analyzed for DM, CP, Ether Extract (EE) and Mineral Matter (MM) according to the Association of Official Analytical Chemists (AOAC, 1990), neutral detergent fiber assayed with heat stable amylase and expressed exclusive of residual ash (aNDFom-NDF), Acid Detergent Fiber expressed exclusive of residual ash (ADFom-ADF) and acid method of fiber analysis. In the aNDFom-NDF analyses, thermostable α-amylase was used without sodium sulfite, using an Ankom® fiber extractor, according to Valente *et al.* (2015). Non-Fiber Carbohydrates (NFCs) in the ingredients of the diets were determined by the following equation:

$$\text{NFC} = 100 - (\% \text{aNDFom-NDF} + \% \text{CP} + \% \text{EE} + \% \text{MM}), \text{ according to Sniffen } et al. (1992).$$

Indigestible aNDFom-NDF was used as an internal marker to estimate pasture intake (Cochran *et al.*, 1986). Preparation of pasture, concentrate, orts and fecal residues from *in situ* incubation followed by aNDFom-NDF extraction was similar to the technique described by Ellis *et al.* (1984) for preparation of indigestible aNDFom-NDF (aNDFom-NDF remaining after 144 h of *in situ* incubation). Fecal output (DMbasis) was calculated from marker concentration using the following formula:

$$\text{Fecal output (g/d)} = \text{Cr dose (mg/d)} / \text{Cr in feces (mg/g feces)}.$$

The contribution of pasture to fecal output was estimated after total fecal output was corrected for contribution of the concentrate by subtracting the indigestible aNDFom-NDF fraction of concentrate from

total fecal output. The indigestible aNDFom-NDF content of pasture and feces was used as an internal marker to estimate intake using the following formula:

$$\text{Pasture DM intake (kg / d)} = \frac{((\text{indigestible aNDFom} - \text{NDF in feces, kg / d}) - (\text{indigestible aNDFom} - \text{NDF in concentrate, kg / d}))}{(\text{indigestible aNDFom} - \text{NDF in forage (kg / kg forage)})}$$

Estimated metabolizable energy and Net Energy of lactation (NEL). For (ME; in Mcal kg<sup>-1</sup> of DM) was

determined according to the National Research Council (NRC, 2001).

The average composition of the pasture in the experimental period is shown in Table 2 and the total diet in Table 3.

In Table 4 shows the analysis of the fatty acid profiles of the concentrate of each diet and in Table 5 is the analysis of the fatty acid profiles of the pasture of the *Cynodon dactylon* (L.) Pers. Cv. Tifton-85.

**Table 2:** Mean chemical composition of pasture (*Cynodon dactylon* (L.) Pers.) as percentage dry matter (DM), organic matter (OM), Crude Protein (CP), Ether Extract (EE), Neutral Detergent fiber (aNDFom-NDF), Acid Detergent Fiber (ADFom-ADF) and Mineral Matter (MM), for Cultivars Coastcross (CC) and Bermudagrass (BG)

Item % <sup>1</sup>	Cultivars <sup>2</sup>	Period 1	Period 2	Period 3	Period 4	Mean ± SD <sup>3</sup>
DM	CC	27.21	25.80	27.42	27.70	27.03±0.85
	BG	27.00	26.79	26.94	27.42	27.04±0.27
OM	CC	92.99	92.16	92.71	93.65	92.88±0.62
	BG	92.07	92.14	92.79	93.28	92.57±0.57
CP	CC	20.59	20.15	23.66	20.76	21.29±1.60
	BG	18.50	18.25	23.71	16.10	19.14±3.23
EE	CC	2.00	2.26	2.08	2.10	2.11±0.11
	BG	2.28	3.20	2.00	1.30	2.20±0.79
aNDFom-NDF	CC	72.24	73.69	70.79	74.70	72.86±1.71
	BG	70.60	70.71	71.22	75.94	72.12±2.56
ADFom-ADF	CC	36.36	36.15	33.64	38.25	36.10±1.89
	BG	35.33	36.68	37.09	36.44	36.39±0.75
MM	CC	7.01	7.84	7.29	6.35	7.12±0.62
	BG	7.93	7.86	7.21	6.72	7.43±0.57

<sup>1</sup>- DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; aNDFom-NDF = neutral detergent fiber; ADFom-ADF = acid detergent fiber; and MM = mineral matter.

<sup>2</sup>- CC = Coastcross; BG = Bermudagrass.

<sup>3</sup>- SD = Standard deviation

**Table 3:** Mean chemical composition of experimental diets for Total Digestibility Nutrient (TDN), Energy Net of lactation (NEL), Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Ether Extract (EE), Neutral Detergent Fiber (aNDFom-NDF), Acid Detergent Fiber (ADFom-ADF), Nonfiber Carbohydrates (NFCs) and Mineral Matter (MM)

	Diets <sup>1</sup>			
	S	SL	SP	SLP
TDN <sup>2</sup> (%)	69.27	70.05	69.79	69.91
NEL <sup>3</sup> (Mcal/kgDM)	1.58	1.59	1.59	1.59
DM (%)	52.98	53.32	54.32	52.31
OM (%)	94.21	93.91	94.03	93.42
CP (%)	22.52	21.72	21.67	21.19
EE (%)	8.02	8.28	8.37	7.36
aNDFom-NDF (%)	52.96	52.87	51.79	53.73
ADFom-ADF (%)	27.14	27.24	27.05	27.26
NFCs <sup>4</sup> (%)	17.41	19.34	19.38	20.36
MM (%)	8.88	8.73	8.92	8.74

<sup>1</sup>Ground sunflower seeds (S); Ground Sunflower seeds with addition of Lignosulfonate (SL); Ground sunflower seeds and pelleted (SP); Ground Sunflower seeds with addition of Lignosulfonate and Pelleted (SLP).

<sup>2</sup>TDN= Total digestible nutrients, estimated in the feed composition according to the NRC (2001), considering that 1 kg of total digestible nutrients (TDNs) contains 4.409 Mcal of Digestible Energy (DE), with the factor 0.82 used in the conversion from DE to metabolizable energy (ME).

<sup>3</sup>NEL = Net energy of lactation. The amount of energy in a feed which is available for milk production and body maintenance.

<sup>4</sup>NFCs = Nonfiber carbohydrates in the ingredients of the diets were determined by the following equation:  $\text{NFC} = 100 - (\% \text{aNDFom} - \text{NDF} + \% \text{CP} + \% \text{EE} + \% \text{MM})$ , according to Sniffen *et al.* (1992).

**Table 4:** Fatty acid profile in concentrate diets; Ground sunflower seeds (S); Ground Sunflower seeds with addition of Lignosulfonate (SL); Ground Sunflower seeds and Pelleted (SP); Ground Sunflower seeds with addition of Lignosulfonate and Pelleted (SLP)

Fatty acid profile	Treatment			
	S	SL	SP	SLP
(% total lipid)				
16:0	8.19	9.09	8.26	8.94
18:0	5.97	6.79	5.92	6.71
18:1n-9	19.55	20.91	20.13	20.10
18:1n-7	0.63	0.68	0.69	0.72
18:2n-6	65.13	61.87	64.45	62.93
18:3n-3	0.53	0.65	0.55	0.59

**Table 5:** Fatty acid profile in pasture of the *Cynodon dactylon* (L.) Pers

Fatty acid profile	Treatment			
	Period 1	Period 2	Period 3	Period 4
(% total lipid)				
16:0	25.49	25.53	25.50	25.51
18:0	11.34	11.35	11.32	11.33
18:1n-9	8.96	8.93	8.97	8.94
18:1n-7	0.98	0.98	1.00	0.99
18:2n-6	14.33	14.34	14.36	14.34
18:3n-3	38.91	38.89	38.88	38.91

### Experimental Procedures

For butter production, 10 liters of milk were collected, proportionally to the morning and afternoon production of each animal. The collected milk was packaged in a plastic bucket and stored at 4°C for 24 h for cream precipitation. After 24 h, the cream was removed and stored in plastic containers for further pasteurization at 75°C for 30 min according to Kazama *et al.* (2010). After pasteurization, the samples were immediately cooled to 4°C for 20 h and beaten in a separate mixer until they turned into butter.

The total fat was extracted with a solution of chloroform-methanol-water (2:2:1.8 v/v/v) according to Bligh and Dyer (1959). Were weighed 15 g of sample in a 250 mL beaker, adding 45.0 mL of chloroform-methanol solution (1:2 v/v) and stirred for about 5 min. Thereafter, were added to the mixture 15.0 mL of chloroform and stirred for 2 min and 18.0 mL of distilled water, stirring for 5 min.

The solution obtained was vacuum filtered on a Büchner funnel with Whatman® quantitative filter paper. The residue retained on the filter paper was transferred to the beaker and added 30 mL of chloroform and 30 mL of H<sub>2</sub>O, then another stirring was performed.

The filtration procedure was repeated, the resulting solution being transferred to a 250 mL separatory funnel. After phase separation, chloroform and fat material were drained to a pre-weighed 250 mL Claisen flask. The solvent is removed on a rotary evaporator under reduced

pressure and kept in water bath in temperature up to 30°C. The solvent residue was discarded with nitrogen flow. The fat material content in the flask was weighed and the lipid content determined gravimetrically.

Fat in butter was separated by centrifugation as described by Murphy *et al.* (1995) and fatty acids in milk fat were methylated according to method 5509 (ISO, 1978) using KOH/methanol (Synth®) and n-heptane (Vetec®). Fatty acid methyl ester profiles were measured at a split ratio of 1:100 by GLC on a Varian chromatograph (Palo Alto, CA, USA) with a G1315A auto sampler equipped with a flame ionization detector and a CP-7420 fused silica capillary column (100 m and 0.25 m film thickness). The column parameters were: Initial column temperature of 65°C was maintained for 4 min; the temperature was then programmed at 20°C/min to 170°C; this temperature was maintained for 22 min and then increased 6°C/min to 235°C and remained at this temperature for 12 min. Injector and detector temperatures were 220 and 240°C, respectively. The carrier gas was hydrogen at 1.2 ml/min. Hydrogen flow to the detector was 30 ml/min, airflow was 300 ml/min and the flow of N<sub>2</sub> make-up gas was 30 ml/min. Fatty acid peaks were identified using pure methyl ester standards of the CLA isomers cis9,trans11-18:2 and trans10,cis12-18:2 (cat#05632; Sigma-Aldrich®) and a commercial mixture of fatty acids (cat#18919; Sigma-Aldrich®). Separations of all fatty acids were obtained in a single chromatographic run. The calculations were performed according to the Joseph and Ackman (1992).

### Statistical Procedures and Model Evaluation

All results were analyzed using the MIXED procedure of SAS (2003) within a 2×2 factorial arrangement of treatments, analyzed using a replicated 4×4 Latin square design with the general model:

$$Y_{ijklm} = \mu + T_i + P_j + S_k + A_l + TS_{ik} + PS_{jk} + A/S_{lk} + e_{ijklm}$$

Where:

- $Y_{ijklm}$  = The dependent variable,  $\mu$  = overall mean
- $T_i$  = Treatment effect (S, SL, SP and SLP)
- $P_j$  = Effect of period (1, 2, 3 and 4)
- $S_k$  = Effect of latin square (1-2)
- $A_l$  = Effect of animal (1-8)
- $TS_{ik}$  = Interaction between the diet and the latin square
- $PS_{jk}$  = Interaction between the period and the latin square
- $A/S_{lk}$  = Animal effect nested inside the latin square
- $e_{ijklm}$  = Residual error

Significance was declared at  $P \leq 0.05$  and a trend was accepted at  $0.05 < P \leq 0.10$ .

### Results

Treatments with pelleted sunflower seeds provided a tendency ( $P = 0.09$ ) to reduce the amounts of 14:1n-7 (3.99 vs 4.44 g/100g of total lipids), while tending to 17:0 (5.70 vs. 5.20 g/100g total lipids) known as margic acid (Table 6).

The fatty acids 16:1n-11, 18:0, 18:1trans (trans-vaccinic acid), 20:0 and CLA 18:2c9t11 had effects according to treatments.

The pelleted diets increased ( $P < 0.05$ ) in the concentrations of fatty acids 16:1n-11 (2.85 vs 1.76), 18:1 trans (87.15 vs 48.03) and CLA 18:2c9t11 (22.75 vs.10.96). However, decreased the amounts of SFA 18:0 (172.92 vs 207.90) e 20:0 (1.74 vs 1.93). No difference for the other fatty acids influenced by diets.

The presence of lignosulfonate in the treatments studied does not affect the composition of fatty acids in butter.

The sum and ratios between groups fatty acids in butter, for the different treatments, is shown in Table 7. Pelleted diets had lower amounts of SFA, CLA total and PUFA (424.05, 12.32, 52.49), respectively, when compared to non-pelleted diets (468.59, 24.15, 60.05).

**Table 6:** Fatty acid profile of butter of cows kept in pasture, supplemented with ground sunflower seeds (S); ground sunflower seeds with addition of lignosulfonate (SL); ground sunflower seeds and pelleted (SP); ground sunflower seeds with addition of lignosulfonate and pelleted (SLP)

Fatty acid profile	Treatment				SE <sup>2</sup>	Probability <sup>1</sup>		
	S	SL	SP	SLP		L	P	I
mg/g total lipid								
4:0	7.03	7.47	7.18	6.07	0.98	0.73	0.53	0.43
6:0	3.73	3.84	4.02	3.31	0.47	0.53	0.79	0.39
8:0	2.54	2.61	2.65	2.23	0.31	0.58	0.67	0.44
10:0	6.03	6.13	6.33	5.28	0.70	0.50	0.70	0.42
12:0	8.46	8.46	9.01	7.87	0.75	0.46	0.98	0.46
14:0	43.99	44.45	45.47	40.75	3.51	0.55	0.76	0.47
14:1n-9	4.83	5.25	4.76	4.57	0.37	0.77	0.32	0.42
14:1n-7	4.63	4.25	4.18	3.80	0.25	0.16	0.09	0.98
15:0	9.41	8.47	9.59	9.03	0.46	0.11	0.43	0.68
15:1n-7	1.78	1.69	1.65	1.55	0.09	0.32	0.17	0.94
16:0	169.64	174.87	160.56	168.04	6.07	0.30	0.20	0.85
16:1n-11	1.87	1.65	2.61	3.09	0.31	0.67	0.01	0.26
16:1n-9	6.07	8.50	7.11	7.43	0.96	0.16	0.99	0.28
16:1n-7	3.16	3.07	3.04	3.08	0.15	0.86	0.71	0.64
17:0	4.98	5.42	5.54	5.85	0.22	0.19	0.07	0.76
17:1n-7	1.59	1.93	1.77	2.05	0.24	0.21	0.55	0.90
18:0	210.83	204.97	172.12	173.72	8.88	0.73	0.01	0.60
18:1trans	51.99	44.06	89.10	85.19	5.88	0.39	0.01	0.73
18:1n-9	315.63	324.69	308.26	323.11	9.45	0.22	0.64	0.76
18:1n-7	2.22	2.98	2.42	2.48	0.36	0.26	0.68	0.34
18:2trans	5.80	5.29	6.14	5.90	0.29	0.22	0.12	0.66
18:2n-6	36.80	35.82	32.99	30.99	2.57	0.65	0.13	0.75
18:3n-6	0.96	0.94	0.96	1.05	0.06	0.52	0.39	0.40
18:3n-3	2.86	2.96	2.88	2.94	0.21	0.70	0.98	0.90
20:0	1.95	1.91	1.76	1.72	0.09	0.66	0.04	0.97
CLA18:2c9t11	11.09	10.83	23.74	21.76	2.22	0.63	0.01	0.72
CLA18:2t10c12	1.37	1.35	1.43	1.35	0.06	0.41	0.66	0.66

<sup>1</sup>- L- Lignosulfonate Effect, P- Pelletizing Effect, I- Interaction.

<sup>2</sup>- SE- Standard error

**Table 7:** The sum and ratios between groups fatty acids in butter of cows kept in pasture, supplemented with ground sunflower seeds (S); ground sunflower seeds with addition of lignosulfonate (SL); ground sunflower seeds and pelleted (SP); ground sunflower seeds with addition of lignosulfonate and pelleted (SLP)

Fatty acid groups <sup>3</sup>	Treatment					Probability <sup>1</sup>		
	S	SL	SP	SLP	SE <sup>2</sup>	L	P	I
mg/g total lipid								
SFA	468.59	468.60	424.23	423.87	9.95	0.97	0.01	0.96
MUFA	341.78	354.01	335.80	351.16	9.93	0.19	0.67	0.88
PUFA	53.08	51.90	62.00	58.09	3.67	0.57	0.05	0.69
Omega 6	37.16	36.77	33.96	32.04	3.62	0.66	0.13	0.77
Omega 3	2.86	2.96	2.89	2.94	0.21	0.70	0.98	0.90
Omega 6/Omega 3	13.22	12.70	12.02	11.28	0.83	0.45	0.12	0.89
CLA total	12.46	12.18	25.18	23.12	2.22	0.60	0.01	0.69

<sup>1</sup>- L- Lignosulfonate Effect, P- Pelletizing Effect, I- Interaction.

<sup>2</sup>- SE = Standard error

<sup>3</sup>- SFA = saturated fatty acid, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids, CLA = conjugated linoleic acid

## Discussion

In a similar study using, lignosulfonate treatment of concentrates containing ground sunflower seeds, Santos *et al.* (2011) observed little effect on milk production and milk composition in our cows which averaged 15.1 kg/d of milk production. This lack of response supports prior observations that treatments such as lignosulfonate have little effect on milk production and composition of low yielding cows. However, cows fed pelleted concentrates had lower milk fat concentration than those fed non-pelleted concentrates, pelleting had no effect on total tract apparent digestibility of nutrients. Pelleting had more effects than lignosulfonate treatment on milk fatty acid profile, with concentrations of trans11-18:1 and CLA, respectively 178% and 200% of those found in milk fat of cows fed non-pelleted concentrates. Pelleting may contribute to improved nutritional value of concentrates containing sunflower seeds as a source of CLA precursors, although there is no additive or synergistic effect between lignosulfonate treatment and pelleting. Similar result found in the present study, when contrasting the different treatments. The pelleting process protects the nutrients of rumen microorganisms and increases the level of non-degradable proteins in the rumen. Heating modifies the amino acid residues of proteins through reactions with other compounds or through cross-linking (Petit *et al.*, 1999). Xylose, which is present in lignosulfonate, can react with several amino groups and this reaction decreases ruminal protein degradation, therefore protecting the proteins from the activity of rumen microorganisms. However, lignosulfonate and pelleting could contribute to release of oils in the rumen, improving the digestibility of nutrients and the milk production of dairy cows. Thus, studies to minimize the negative effects of high fat addition and increase PUFA uptake in milk are important to improve the impact of sunflower seeds use on ruminant nutrition (Marchi *et al.*, 2013).

According to Table 4, the composition in the fatty acids of the treatments is characteristic of diets containing sunflower seeds. With a mean of 63.59% of 18:2n-6 and the lipid composition of the butter of the cows were similar the fatty acids presents into the milk and kept after the butter was produced. Figueiroa *et al.* (2013) pelleted concentrates containing sunflower seeds can be used for producing milk with a lower fat content in Holstein cows. Although, the pelleting process or lignosulfonate addition are not effective in protecting the Unsaturated Fatty Acids (UFAs) of the diet or improving the milk fatty acid and butter texture. Middaugh *et al.* (1988) tested the use of diets based on sunflower seeds fed to Holstein cows and observed an increase in the UFAs content in milk fat and, consequently, a higher amount of these fatty acids in the butter.

The presence of lignosulfonate in the treatments studied does not affect the composition of fatty acids in butter. Neves *et al.* (2007) feeding cows with extruded versus non-extruded soybeans, observed decreased milk fat concentration and concentrations of SFA in milk fat, while it increased concentrations of CLA in milk fat. Concentrations of cis9, trans11 CLA and PUFA tended to be increased by lignosulfonate treatment of full-fat soybeans. But that lignosulfonate treatment has less important effects on the milk fatty acid profile, similar result found in the present study.

The lignosulfonate protects fatty acids against ruminal biohydrogenation (Wright *et al.*, 2005; Neves *et al.*, 2009) and may alter the composition of fatty acids in butter (Santos *et al.*, 2011). It is possible that an expressive amount of UFAs present in the pelleted treatments occurred partial biohydrogenation in the rumen observed in butter, higher amounts of intermediates of this process, such as 18:1 trans and trans 11, cis9-18:2 (CLA). According to Harfoot and Hazlewood (1997) the final result of ruminal biohydrogenation is stearic acid. This process can be considered a barrier in the incorporation of unsaturated fatty acids to the milk and its derivatives.

However, according to several research results it is possible to reduce the effects of bacteria on the UFAs contained in the diet, through chemical or physical processing (Chouinard *et al.*, 1997; Silva *et al.*, 2007). With the results obtained in this experiment, pelleting can reduce biohydrogenation of fatty acids. The increase of one of the intermediates of this process, 18: 1 trans, is one of the reasons that can justify this assertion, since this intermediate was found almost double the amount, in the butter of the cows that received the pelleted diets. The higher amounts of trans11, cis9-18: 2 (CLA) in the butter of animals receiving heat-treated diets are extremely interesting results. For the health of the consumed this butter is relevant, since CLA has been related to anticarcinogenic, antiatherogenic effects, increase of the immune response, reduction of accumulated fat in the body and also, antidiabetic effect (Whigham *et al.*, 2000; Tanaka, 2005).

The synthesis of conjugated linoleic acid occurs with the presence of the Delta 9-desaturase enzyme in the mammary gland. The precursor is 18: 1 trans, intermediate of the biohydrogenation of linoleic and  $\alpha$ -linolenic acids (Tanaka, 2005). The values found for trans11, cis9-18: 2 in higher quantity with pelleting may be the greater amount of trans 18:1 (twice more) available in the mammary gland. Increases in 18: 1 trans and CLA (all isomers) in butter when they provided sunflower co-products in the diet of lactating cows when compared to the control treatment (Bauman *et al.*, 1999).

Diet rich in oleaginous grains may increase ruminal biohydrogenation, consequently the presence of SFA. However, by using protective techniques of unsaturated fatty acids present in the diet, against this process, it is possible to incorporate these in the products of ruminants (meat, milk and dairy products).

The animals that received pelleted diets had higher PUFA values and lower SFA values. The results obtained in this experiment confirm those found by Bauman *et al.* (1999).

The composition of the fatty acids of the butter can alter its characteristics, mainly of texture, in a greater amount of UFAs responsible for a softer and lighter color butter (Cavaliere *et al.*, 2005). In these experiments fat-rich PUFA were provided to dairy cows for the production of butter, this fact is similar to the present study because the sunflower is an oleaginous rich in UFAs and also present in butter. Bett *et al.* (2005) also found PUFA values higher than SFA, in a study with dairy cows fed diets with sunflower seeds.

CLA corresponds to the group of linoleic acid isomers which are present in foods produced by ruminants. CLA presents important nutraceutical properties and add greater nutritional value and consequent improvement of human health (Barendse, 2014). The MUFA and PUFA are directly related to a healthy diet of humans, since, balanced human diets are

recommended the ingestion of more UFAs (omega 3 and CLA) (Lima *et al.*, 2017). The consumption of foods rich in UFAs, such as butter, may be responsible for the reduction in the incidence of cardiovascular diseases (Sangiovanni *et al.*, 2000; Bucher *et al.*, 2002; Lorgieril and Salen, 2002), prevention of arteriosclerosis and thrombosis, resulting from the modification of lipid metabolism (Petit, 2002).

## Conclusion

Diets with pelleted sunflower seeds modify the fatty acid composition of butter and increase CLA18:2c9t11.

The heat treatment used in pelleted diets reduces the amount of SFA and increases PUFA.

Diets with lignosulfonate do not modify the fatty acid profile in butter.

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## Author's Contributions

**Wallacy Barbacena Rosa dos Santos, Daniele Cristina da Silva Kazama, Johnny Paulo Monteiro and Francilaine Eloise De Marchi:** designed and carried out the experiment.

**Geraldo Tadeu dos Santos, Tiago Neves Pereira Valente andréia Santos Cezário and Jeferson Corrêa Ribeiro:** Critically analysed the manuscript and provided guidance. Additionally, the authors have read and approved the final version of this manuscript.

## Conflict of interest

The authors declare that they have no conflict of interest related to this study.

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## Abbreviations

ADFom-ADF, acid detergent fiber expressed exclusive of residual ash; aNDFom-NDF, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; CLA, conjugated linoleic acid; CP, crude protein; DM, dry matter; EE, ether extract; MM, mineral matter; MUFA, monounsaturated fatty acids; NFCs, nonfiber carbohydrates; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TDN, total digestible nutrients. UFAs, unsaturated fatty acids.