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# Production, composition and fatty acid profile of milk and butter texture of dairy cows fed ground or pelleted concentrate with sunflower and/or lignosulfonate[1]

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**ABSTRACT** - The objective of this study was to evaluate the milk production, composition, milk fatty acid profile, butter texture and blood parameters of Holstein cows fed corn silage and concentrate containing one of the following: ground sunflower seeds; ground sunflower seeds treated with 50 g of lignosulfonate/kg of sunflower dry matter; pelleted sunflower seeds; or ground sunflower seeds pelleted and treated with 50 g of lignosulfonate/kg of sunflower dry matter. Four lactating cows were used, each with 130±28 days in lactation and a body weight of 569±63 kg. These animals were distributed in a Latin square design with four periods of 21 days each, with 14 days of adaptation and seven days of data collection. The diets were formulated to meet nutritional requirements and had a forage:concentrate ratio of 60:40. The milk fat was lower in the pelleted treatments. The concentrations of 16:1 n-11 and trans18:1 n-9 in the milk increased, and the n-6:n-3 ratio was higher for the pelleted treatments. The firmness and adhesiveness of the butter and the blood parameters analyzed were not affected by the treatments. Addition of lignosulfonate is not effective in protecting polyunsaturated fatty acids from the ruminal bio hydrogenation process, and the pelleting process has little effect on the milk fatty acid profile and can not change the butter texture.

Key Words: biohydrogenation, fat, firmness, metabolite, oilseed

## Introduction

There is a growing demand for foods that, in addition to being nutritious, can provide other benefits to human health. Therefore, research has been conducted aiming at increasing the amount of substances beneficial to human health in foods, such as omega 3, omega 6 and conjugated linoleic acids (CLA) and, at the same time, reducing the levels of harmful substances, such as saturated fatty acids (SFA) in meat and milk, to enable ingestion of the recommended daily amounts of these nutrients (Nutrition and Health Collection, 1999; Parodi et al., 1999; Hennessey et al., 2011).

To improve the milk fatty acid profile and dairy products, the supply of fat sources rich in omega 3 and omega 6, such as oilseeds, in the diet of dairy cows has shown to be efficient (Bett et al., 2005; Rego et al., 2005; Neves et al., 2009). The oil content in sunflower seeds varies by cultivar from 20 to 45%, with a composition of 11% SFA, 27% monounsaturated (MUFA) and 60% polyunsaturated (PUFA) fatty acids, of which 60.5% is linoleic acid (Corsini et al., 2008).

In ruminants, the appearance of PUFA in milk may be compromised by the biohydrogenation process performed by rumen microorganisms as a defense against the toxicity of these fats. In an attempt to reduce the biohydrogenation process of some products, some processes, such as pelletization and addition of lignosulfonate have been adopted (Petit et al., 1999; Neves et al., 2007).

Pelleting makes food denser, reduces the selectivity and segregation of nutrients, destroys pathogens and makes the food more palatable, reducing the presence of dust particles and facilitating ingestion (Behnke, 1996; Chouinard et al., 1997). Pelletization is a type of physical processing that involves humidity and heat. According to Kennelly et al. (1996), heat treatments can reduce the extent of the ruminal biohydrogenation of PUFA. Lignosulfonate is a byproduct of wood processing and contains a variety of sugars, especially xylose, which have binding, wetting and other properties. According to Petit et al. (1999), the addition of this product to the diets of dairy cows can decrease the rumen degradability of some nutrients.

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Unsaturated fat is known to be liquid at room temperature, and the butter produced from milk enriched with this type of fat does not have the fatty acid content modified (Byers & Schelling, 1993; Van Soest, 1994). Thus, improving the softness of butter may be possible. Therefore, the objective of this study was to evaluate the effect of pelleting and lignosulfonate addition on the production, composition and fatty acid profile of milk from cows fed diets with sunflower seeds and to determine whether the butter firmness and stickiness were affected by this type of diet.

## Material and Methods

For this work, four multiparous Holstein cows averaging 569±63 kg of body weight (BW), 17±0.7 kg of milk/d and 130±28 d in milk were assigned to a 4 × 4 Latin square design to determine the effects of pelleting and lignosulfonate treatment of sunflower seeds on the DM intake, milk production, milk composition, milk fatty acid profile, butter texture and blood parameters. Each experimental period consisted of 14 d of adaptation to the diets and 7 d for data gathering.

The cows were confined in individual stalls, and feed was provided twice a day at 07h15 and 16h00, immediately after milking. The animals received 70% of the diet in the morning and 30% in the afternoon. The amount provided to each animal was set to have 10% remaining. The ratio of corn silage and feed concentrate was 60:40 (Table 1), which was calculated to meet the nutritional requirements of dairy cows according to the NRC (2001). The feed concentrates were offered directly in the trough and mixed with the silage.

The feed concentrates studied were as follows (Table 1): ground sunflower seeds (GS); ground sunflower seeds treated with 50 g of lignosulfonate/kg of sunflower DM (GSL); pelleted sunflower seeds (PS); and ground sunflower seeds pelleted and treated with 50 g of lignosulfonate/kg of sunflower DM (GSL). The lignosulfonate solution was prepared with Lignosol (Melbar, São Paulo, SP, Brazil) and contained 27 g/kg DM of xylose. The lignosulfonate solution was added at 50 g/kg DM after grinding the seeds but before pelleting the concentrates, as a similar concentration of lignosulfonate was shown to decrease the CP degradability and increase the milk yield (Wright et al., 2005). In the GS and PS treatments, nothing was added to substitute for the lignosulfonate.

The pelleting of the concentrates was performed with a 40 HP pelleting machine (Indústria e Comércio Chavantes Ltda, Chavantes, SP, Brazil) without steam addition at a 75 ºC exit temperature. The 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.

Milk production was recorded at every milking. Milk samples were obtained from the four consecutive milkings on days 15 and 16 of each experimental period and pooled within the cow and period, weighted to production, to obtain one composite milk sample/cow/period for the chemical analysis. The milk samples were kept at room temperature (i.e., 23 ºC) with preservative 2-bromo-2-nitropropane-1,3 diol (Bronopol, D&F Control Systems Inc., San Ramon, CA, USA) for determination of CP, fat, lactose, total solids and somatic cell count concentrations. One sample from each sampling day without preservative was kept frozen to determine the milk fatty acid profile and milk urea N concentration. The determination of milk acidity and density was performed immediately after collection using the Dornik solution and a thermolactodensimeter, respectively.

The N, fat, total solid and lactose concentrations in milk were determined by infrared spectroscopy (Bentley model 2000; Bentley Instrument, Inc., Chaska, MN, USA) following procedure 972.16 of the AOAC (1990). The

Table 1 - Ingredient and chemical composition of total mixed diets

| | Diets | | | |
|---|---|---|---|---|
| | GS | GSL | PS | PSL |
| Ingredient (g/kg DM) | | | | |
| Corn silage | 600.0 | 600.0 | 600.0 | 600.0 |
| Ground corn grain | 52.7 | 52.7 | 52.7 | 52.7 |
| Soybean meal | 185.8 | 185.8 | 185.8 | 185.8 |
| Mineral and vitamin supplement[1] | 18.0 | 18.0 | 18.0 | 18.0 |
| Limestone | 10.6 | 10.6 | 10.6 | 10.6 |
| Magnesium oxide | 2.8 | 2.8 | 2.8 | 2.8 |
| Salt | 6.1 | 6.1 | 6.1 | 6.1 |
| Sunflower seeds | 134.0 | 134.0 | 134.0 | 134.0 |
| Lignosulfonate (of sunflower DM) | - | 50 | - | 50 |
| Chemical analyses | | | | |
| Dry matter (g/kg fresh weight) | 547.5 | 545.9 | 544.7 | 544.0 |
| Organic matter (g/kg DM) | 937.6 | 937.9 | 937.4 | 936.7 |
| Crude protein (g/kg DM) | 181.0 | 178.4 | 177.9 | 172.7 |
| Ether extract (g/kg DM) | 71.3 | 65.3 | 71.7 | 69.2 |
| Neutral detergent fiber (g/kg DM) | 459.5 | 450.8 | 452.8 | 445.2 |
| Acid detergent fiber (g/kg DM) | 271.5 | 268.2 | 268.8 | 265.7 |
| Mineral matter (g/kg DM) | 62.4 | 62.1 | 62.6 | 63.3 |
| C16:0 | 8.49 | 9.65 | 8.31 | 8.46 |
| C18:0 | 7.11 | 6.15 | 6.08 | 5.49 |
| C18:1 n9 | 22.41 | 21.34 | 21.91 | 21.12 |
| C18:2 n6 | 60.88 | 61.38 | 62.44 | 64.40 |
| C18:3 n3 | 1.12 | 1.50 | 1.29 | 0.78 |

GS - ground sunflower seeds; GSL - ground sunflower seeds with 50 g of lignosulfonate/kg of sunflower DM; PS - pelleted sunflower seeds; PSL - pelleted sunflower seeds treated with 50 g of lignosulfonate/kg of sunflower DM.
C16:0 - palmitic acid; C18:0 - stearic acid; C18:1 n9 - oleic acid; C18:2 n6 - linoleic acid; C18:3 n3 - α-linolenic acid.
[1] Ca - 270 g/kg; P - 80 g/kg; S - 20 g/kg; Mg - 15 g/kg; Fe - 2,200 mg/kg; Cu - 800 mg/kg; Co - 50 mg/kg; I - 60 mg/kg; Se - 40 mg/kg; Zn - 2,800 mg/kg, Mn - 2,680 mg/kg; vit. A - 216,000 IU/kg; vit. D - 67,600 IU/kg; vit. E - 500 mg/kg.

concentrations of milk urea N were determined according to Marsh et al. (1965). The milk somatic cell counts (SCC) were obtained using an electronic counter (Somacount 500®, Chaska, MN, USA) as described by Voltolini et al. (2001).

The milk fat was separated by centrifugation as described by Murphy et al. (1995), and the milk fatty acids were methylated according to method 5509 (ISO, 1978) using KOH/methanol (Synth®, São Paulo, Brazil) and n-heptane (Vetec®, Rio de Janeiro, Brazil).

The methyl ester fatty acid 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 mm i.d., 0.25 µm film thickness). The column parameters were as follows: the initial column temperature of 65 ºC was maintained for 4 min; the temperature was then programmed to 20 ºC/min until 170 ºC; the temperature of 170 ºC was maintained for 20 min and then increased by 6 ºC/min to 235 ºC; and the temperature of 235 ºC was maintained for 14 min. The injector and detector temperatures were 220 ºC and 240 ºC, respectively. The carrier gas was hydrogen at 1.2 mL/min. The hydrogen flow to the detector was 30 mL/min, the airflow was 300 mL/min, and the flow of N2 make-up gas was 32 mL/min. The 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 Brazil Ltd., São Paulo, SP, Brazil) and a commercial mixture of fatty acids (cat#18919; Sigma–Aldrich Brazil Ltd.). The separations of all fatty acids were obtained in a single chromatographic run.

On the last morning of each experimental period, blood samples were collected from the animals' coccygeal vein before morning feeding. The blood samples were centrifuged at 3200 rpm for 20 minutes, and the plasma was separated, placed in Eppendorf vials and stored at −20 °C, using methods described by Cavalieri (2003). The analyzed blood parameters were VLDL (very low density lipoprotein), LDL (low-density lipoprotein), HDL (high-density lipoprotein), total cholesterol (enzymatic photometric test), triglycerides (enzymatic colorimetric test) and glucose (enzymatic colorimetric test) on the Vitalab Selectra 2 with commercial Kits (Diasys®).

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 hours for cream precipitation. After 24 hours, the cream was removed and stored in plastic containers for further pasteurization at 75 °C for 30 minutes. After pasteurization, the samples were immediately cooled to 4 °C for 20 hours and beaten in a separate mixer until they turned into butter.

The butter texture analyses were made using a 45° conical probe in a texture measurer TA.XT *plus* Texture Analyzer (Stable Micro Systems, London, UK). The probe penetrated 23 mm from the sample surface at a speed of 3 mm/s, where the penetration force applied on the sample was reported as the firmness of the butter, and the negative force applied to remove the probe was reported as the adhesion.

Statistical analyses were performed using the PROC MIXED procedure of SAS (Statistical Analysis System, version 9.0), with a 2 × 2 factorial arrangement.

The statistical model was $Y_{ijk} = \mu + T_i + P_j + Ak + e_{ijk}$, where $Y_{ijm}$ = the observation on the repetition m for the treatment i in the period j; $\mu$ = the overall mean; $T_i$ = the treatment effect i (GS, GSL, PS, PSL); $P_j$ = the period effect j (1, 2, 3 and 4); Ak = the animal effect (1, 2, 3 and 4); and $e_{ijk}$ = the random error associated with each observation m, receiving treatment i in period j. The treatments were compared to provide the factorial contrasts: pelleted *versus* non-pelleted, with lignosulfonate *versus* without lignosulfonate, and the interaction between pelleted and lignosulfonate. Significance was declared for $P<0.05$, and tendency was accepted for $P<0.10$.

## Results

The milk production and fat-corrected milk yield were not affected by the diet. The content of protein, lactose, fat and total solids were not affected by the lignosulfonate (Table 2). The protein concentration, lactose, total solid, and fat yields were also not affected by the diet. However, milk from cows treated with the pelleted concentrate had a lower fat content (P = 0.455) than milk from cows treated with the non-pelleted concentrate (2.72 × 3.13%).

The milk from cows fed lignosulfonate had a tendency to have increases in the concentrations of C16:1n11 (P = 0.069) and C16:1n7 (P = 0.053) in the fatty acid composition (Table 3). The concentrations of C16:1n11 (P = 0.033) and C18:1n9 trans (P = 0.030) were higher in the milk from cows fed the pelleted diets. Moreover, a tendency was observed in the interaction effect between the lignosulfonate addition and pelleting process to decrease the C11:0 (P = 0.083) and C14:1n7 (P = 0.803) fatty acids, comparing the PSL with the GS.

There were no significant effects of the experimental diets on the total saturated, monounsaturated, and polyunsaturated fatty acids or of short-, medium- and long-chain fatty acids. In addition, the levels of omega 3 and

omega 6 were not affected by the diets (Table 4). However, the omega 6:omega 3 ratio was higher (P = 0.043) in the milk from cows fed PS and PSL compared with the non-pelleted diets (21.2 × 17.6).

In the blood parameters (Table 5), the total cholesterol and LDL levels were numerically lower in the PSL diet, but no significant difference was observed among the diets. The levels of glucose, HDL, VLDL, triglycerides and urea in the blood plasma (Table 5) were also not affected by the experimental diets.

The means of the glucose, total cholesterol, HDL, LDL, VLDL, triglycerides and urea were 64.79, 181.31, 92.5, 84.89, 3.93, 19.63 and 29.25 mg/dL, respectively.

Finally, no effect of the diets was observed on the firmness and stickiness of the butter (Table 6). The average butter firmness and stickiness were 16.03 and -8.236 g, respectively.

Table 2 - Production and milk composition of Holstein cows fed the different treatments

| Variables | Treatment | | | | SE | Probability | | |
|---|---|---|---|---|---|---|---|---|
| | GS | GSL | PS | PSL | | L | P | I |
| Dry matter intake (kg/day) | 15.50 | 16.35 | 16.25 | 15.64 | 2.04 | 0.956 | 0.992 | 0.727 |
| Milk production (kg/day) | 18.18 | 17.63 | 16.85 | 16.70 | 2.80 | 0.903 | 0.696 | 0.944 |
| CMY (kg/day) | 15.52 | 15.25 | 13.90 | 13.27 | 2.31 | 0.849 | 0.455 | 0.940 |
| Fat (g/100 g) | 3.07 | 3.18 | 2.81 | 2.63 | 0.12 | 0.778 | 0.007 | 0.241 |
| Protein (g/100 g) | 3.28 | 3.33 | 3.27 | 3.34 | 0.25 | 0.827 | 0.992 | 0.976 |
| Lactose (g/100 g) | 4.36 | 4.44 | 4.32 | 4.36 | 0.16 | 0.728 | 0.716 | 0.895 |
| Total solids (g/100 g) | 11.63 | 11.88 | 11.83 | 11.33 | 0.26 | 0.709 | 0.162 | 0.580 |
| Fat production (g/100 g) | 0.55 | 0.55 | 0.48 | 0.44 | 0.08 | 0.812 | 0.312 | 0.835 |
| Protein production (g/100 g) | 0.60 | 0.56 | 0.55 | 0.54 | 0.07 | 0.798 | 0.671 | 0.825 |
| Lactose production (kg) | 0.80 | 0.79 | 0.74 | 0.74 | 0.14 | 0.993 | 0.723 | 0.965 |
| Total solids (kg) | 2.11 | 2.06 | 1.93 | 1.90 | 0.32 | 0.890 | 0.599 | 0.975 |
| Somatic cell score[1] | 2.70 | 2.97 | 2.82 | 2.98 | 0.22 | 0.336 | 0.762 | 0.813 |
| Nitrogen urea (mg/dL) | 14.39 | 14.42 | 13.52 | 13.08 | 0.84 | 0.808 | 0.219 | 0.784 |

GS - ground sunflower seeds; GSL - ground sunflower seeds with 50 g of lignosulfonate/kg DM sunflower; PS - pelleted sunflower seeds; PSL - pelleted sunflower seeds treated with 50 g of lignosulfonate/kg DM sunflower.
SE - standard error; L - lignosulfonate effect; P - pelleted effect; I - interaction effect.
CMY - 4% fat-corrected milk yield [0.4 × milk yield (kg/day) + 15 × fat production (kg/day)], (Gravert, 1987).
[1]Log10 somatic cell count.

Table 3 - Milk fatty acid profile (g/100 g of fatty acids) of Holstein cows fed the different treatments

| Fatty acids | Treatment | | | | SE | Probability | | |
|---|---|---|---|---|---|---|---|---|
| | GS | GSL | PS | PSL | | L | P | I |
| C4:0 | 1.08 | 0.90 | 0.90 | 0.92 | 0.17 | 0.683 | 0.617 | 0.548 |
| C6:0 | 0.75 | 0.64 | 0.57 | 0.61 | 0.10 | 0.745 | 0.324 | 0.450 |
| C8:0 | 0.50 | 0.42 | 0.38 | 0.40 | 0.06 | 0.608 | 0.232 | 0.333 |
| C10:0 | 1.37 | 1.14 | 1.02 | 1.09 | 0.12 | 0.525 | 0.124 | 0.241 |
| C11:0 | 0.11 | 0.09 | 0.07 | 0.09 | 0.01 | 0.891 | 0.083 | 0.074 |
| C12:0 | 1.80 | 1.55 | 1.48 | 1.61 | 0.11 | 0.591 | 0.277 | 0.121 |
| C14:0 | 8.10 | 7.44 | 7.26 | 7.74 | 0.54 | 0.882 | 0.632 | 0.322 |
| C14:1n11 | 0.20 | 0.17 | 0.16 | 0.18 | 0.02 | 0.912 | 0.677 | 0.363 |
| C14:1n9 | 0.52 | 0.53 | 0.48 | 0.62 | 0.05 | 0.141 | 0.598 | 0.227 |
| C14:1n7 | 0.39 | 0.31 | 0.32 | 0.36 | 0.03 | 0.643 | 0.803 | 0.092 |
| C15:0 | 0.62 | 0.59 | 0.60 | 0.62 | 0.05 | 0.952 | 0.874 | 0.679 |
| C15:1n7 | 0.27 | 0.24 | 0.28 | 0.33 | 0.07 | 0.890 | 0.487 | 0.550 |
| C16:0 | 20.21 | 21.43 | 20.46 | 21.30 | 1.06 | 0.354 | 0.955 | 0.859 |
| C16:1n11 | 0.09 | 0.12 | 0.13 | 0.22 | 0.03 | 0.069 | 0.033 | 0.267 |
| C16:1n9 | 0.12 | 0.14 | 0.14 | 0.21 | 0.03 | 0.135 | 0.152 | 0.360 |
| C16:1n7 | 1.05 | 1.33 | 1.15 | 1.27 | 0.09 | 0.053 | 0.793 | 0.415 |
| C17:0 | 0.35 | 0.31 | 0.36 | 0.38 | 0.03 | 0.684 | 0.228 | 0.300 |
| C17:1n7 | 0.30 | 0.30 | 0.34 | 0.31 | 0.03 | 0.637 | 0.416 | 0.681 |
| C18:0 | 18.80 | 18.60 | 18.39 | 16.03 | 0.90 | 0.189 | 0.132 | 0.259 |
| C18:1n9 trans | 4.27 | 5.50 | 6.12 | 7.13 | 0.68 | 0.132 | 0.030 | 0.876 |
| C18:1n9 | 33.67 | 32.74 | 33.18 | 31.76 | 1.98 | 0.566 | 0.719 | 0.906 |
| C18:2n6 trans | 0.42 | 0.46 | 0.42 | 0.48 | 0.05 | 0.390 | 0.922 | 0.842 |
| C18:2n6 | 3.71 | 3.74 | 4.29 | 4.40 | 0.46 | 0.873 | 0.207 | 0.938 |
| C18:3n3 | 0.23 | 0.24 | 0.24 | 0.22 | 0.01 | 0.785 | 0.64 | 0.336 |
| C18:2 c9,trans11[1] | 1.09 | 1.08 | 1.32 | 1.66 | 0.37 | 0.664 | 0.304 | 0.641 |

GS - ground sunflower seeds; GSL - ground sunflower seeds with 50 g of lignosulfonate/kg DM sunflower; PS - pelleted sunflower seeds; PSL - pelleted sunflower seeds treated with 50 g of lignosulfonate/kg DM sunflower.
SE - standard error; L - lignosulfonate effect; P - pelleted effect; I - interaction effect.
[1] Conjugated linoleic acid (18:2 isomer cis-9, trans-11).

Table 4 - Composition and ratio (g/100 g) of milk fatty acids of Holstein cows fed the different treatments

| Fatty acids | Treatment | | | | SE | Probability | | |
|---|---|---|---|---|---|---|---|---|
| | GS | GSL | PS | PSL | | L | P | I |
| Saturated | 53.68 | 53.11 | 51.47 | 50.80 | 2.25 | 0.788 | 0.340 | 0.983 |
| Monounsaturated | 40.87 | 41.37 | 42.28 | 42.40 | 2.02 | 0.881 | 0.559 | 0.927 |
| Polyunsaturated | 5.45 | 5.53 | 6.27 | 6.76 | 0.64 | 0.666 | 0.144 | 0.749 |
| Omega 6 | 4.13 | 4.21 | 4.71 | 4.88 | 0.47 | 0.800 | 0.218 | 0.922 |
| Omega 3 | 0.23 | 0.24 | 0.24 | 0.22 | 0.01 | 0.785 | 0.624 | 0.336 |
| Omega 6:omega 3[1] | 17.72 | 17.47 | 20.1 | 22.34 | 1.54 | 0.535 | 0.043 | 0.440 |
| Short-chain | 5.61 | 4.73 | 4.39 | 4.72 | 0.52 | 0.607 | 0.268 | 0.276 |
| Medium-chain | 31.55 | 32.30 | 30.98 | 32.86 | 1.52 | 0.407 | 0.996 | 0.717 |
| Long-chain | 62.84 | 62.97 | 64.65 | 62.39 | 1.87 | 0.582 | 0.752 | 0.539 |

GS - ground sunflower seeds; GSL - ground sunflower seeds with 50 g of lignosulfonate/kg DM sunflower; PS - pelleted sunflower seeds; PSL - pelleted sunflower seeds treated with 50 g of lignosulfonate/kg DM sunflower.
SE - standard error; L - lignosulfonate effect; P - pelleted effect; I - interaction effect.
[1] Ratio between the total omega 6 and omega 3 fatty acids.

Table 5 - Blood parameters of Holstein cows fed the different treatments

| Metabolites (mg/dL) | Treatment | | | | SE | Probability | | |
|---|---|---|---|---|---|---|---|---|
| | GS | GSL | PS | PSL | | L | P | I |
| Glucose | 64.5 | 64.75 | 65.25 | 64.75 | 2.03 | 0.952 | 0.857 | 0.857 |
| Total cholesterol | 182.5 | 199.75 | 185.75 | 157.25 | 24.58 | 0.824 | 0.445 | 0.376 |
| High-density lipoprotein | 89.75 | 95.00 | 94.50 | 90.75 | 4.60 | 0.874 | 0.957 | 0.353 |
| Low-density lipoprotein | 89.85 | 100.80 | 86.85 | 62.05 | 21.83 | 0.758 | 0.364 | 0.434 |
| Very low-density lipoprotein | 2.90 | 3.95 | 4.40 | 4.45 | 1.48 | 0.718 | 0.516 | 0.743 |
| Triglycerides | 14.50 | 19.75 | 22.00 | 22.25 | 7.40 | 0.718 | 0.516 | 0.743 |
| Urea | 29.00 | 32.75 | 27.25 | 28.00 | 1.98 | 0.284 | 0.134 | 0.467 |

GS - ground sunflower seeds; GSL - ground sunflower seeds with 50 g of lignosulfonate/kg DM sunflower; PS - pelleted sunflower seeds; PSL - pelleted sunflower seeds treated with 50 g of lignosulfonate/kg DM sunflower.
SE - standard error; L - lignosulfonate effect; P - pelleted effect; I - interaction effect.

Table 6 - Firmness and adhesiveness of the butter from the milk of Holstein cows fed the different treatments

| Texture (g) | Treatment | | | | SE | Probability | | |
|---|---|---|---|---|---|---|---|---|
| | GS | GSL | PS | PSL | | L | P | I |
| Firmness | 18.17 | 15.77 | 14.84 | 15.32 | 2.36 | 0.694 | 0.445 | 0.557 |
| Adhesiveness | −8.474 | −8.474 | −7.970 | −8.026 | 725 | 0.970 | 0.527 | 0.970 |

GS - ground sunflower seeds; GSL - ground sunflower seeds with 50 g of lignosulfonate/kg DM sunflower; PS - pelleted sunflower seeds; PSL - pelleted sunflower seeds treated with 50 g of lignosulfonate/kg DM sunflower.
SE - standard error; L - lignosulfonate effect; P - pelleted effect; I - interaction effect

## Discussion

Processes such as pelleting and lignosulfonate addition in ruminant diets are performed to protect the nutrients from rumen degradation, including PUFA and protein (Petit et al., 1999; Wernersbach Filho et al., 2006). The increase in ruminal undegradable protein (RUP) is a factor that can increase milk production (Wright et al., 2005). One objective of the use of lignosulfonate and pelleting in the present study was to increase milk production through the possible protection of true protein and fat, performed by these treatments; however, this effect was not observed. Nonetheless, Santos et al. (2006) stated that gains in milk production from sources high in RUP are more likely in cows with production greater than 30 kg milk/day.

Similar results were also observed in other studies conducted with oilseeds and heat processing. For example, Neves et al. (2009) worked with extruded canola seeds with and without 50 g/kg DM of lignosulfonate addition and found no significant effect on the milk production and composition. However, working with extruded soybean treated with 30 g of lignosulfonate/kg DM, these same authors (Neves et al., 2007) observed a tendency to increase milk production when the animals received the extruded treatments.

The animals used in this study and those used in the studies by Neves et al. (2007) and Neves et al. (2009) were of average production: 17, 21 and 19 kg milk/day, respectively. Therefore, gains were more difficult to obtain. In agreement with the above findings, Wright et al. (2005),

who worked with high-producing cows (36 kg milk/day) and evaluated the effect of diets containing untreated canola seeds, seeds treated with moist heat (extrusion) or seeds treated with wet heat and with lignosulfonate addition, observed an increase in milk production of cows fed the canola treated with heat and lignosulfonate compared with the untreated diet (36.6 × 34.8 kg/day). In addition to the higher milk production of the cows used, the temperature during the extrusion process was greater than that used herein in the pelleting process.

With respect to the milk composition, similar results were also observed by Neves et al. (2007), except for the lactose level, which was increased in the milk of cows receiving treatments based on inclusion of lignosulfonate with extruded soybeans. Wright et al. (2005) also observed no differences in the milk chemical composition, except for the milk urea nitrogen (MUN) concentration, which was lower in milk from cows fed the treated canola. A reduction in the fat content in milk from the cows treated with pelleted diets was also observed by Moalem et al. (2009), who worked with thermal processing (extrusion) in the diet of dairy cows. However, the authors observed higher milk production in the cows fed extruded diets.

Fat inclusion in the diets, especially at levels above 7%, can compromise the fiber degradability due to a combination of factors, including the formation of a physical barrier that coats the fiber, hindering the action of microorganisms in the rumen, and the toxicity of PUFA to the gram-positive bacteria (Palmquist, 1989; Jenkins, 1993). There are alternative methods to make the fat inert in the ruminal environment, to protect the unsaturated fatty acids and to incorporate the unsaturated fatty acids into the milk, such as the use of calcium salts and heat treatments. However, in the present work, the lignosulfonate addition and pelleting process were not efficient to accomplish these outcomes.

The reduction of fiber degradation can reduce the levels of ruminal acetic acids and, consequently, the fat content in milk. However, it was no possible to determine whether the fat content of the diet reduced the fat content in the milk because all diets had, on average, the same fat content (7%). In the present study, the pelleting process was not efficient in protecting and improving the levels of PUFA in the milk but was efficient in reducing the fat content of milk (Tables 3 and 4). One possible explanation is that the temperature during the pelleting process may have been sufficient to rupture the fat globules, exposing the PUFA to the ruminal digestion, and yet it may have been insufficient to bind the fat with other nutrients, thus promoting the protection of the fatty acids.

This release of PUFA in the rumen may have provided an increased formation of specific isomers of C18:1 trans, which were actually higher in the pelleted diets and could be related to the milk fat decrease (Baumgard et al., 2000; Dhimam et al., 2000; Pirepova et al., 2000). According to Bauman & Griinari (2003) and Harvatine & Allen (2006), milk fat reduction is associated with incomplete polyunsaturated fatty acid biohydrogenation, which stimulates the duodenal flow of trans C18:1 and C18:2 10t 12c and impacts the reduction of genes related to lipogenesis.

The increase in the levels of C18:1 n9 trans in the milk fat of animals that were fed pellets may explain the reduction in the milk fat of these animals. Baumgard et al. (2000) concluded that the isomer C18:2 10t, 12c (CLA) is the isomer responsible for milk fat depression, but this isomer was not identified in the present study.

The results for omega 3 and omega 6 are in agreement with those of Neves et al. (2009). The increase in the omega 6:omega 3 ratio may be due to an increased ruminal release of fatty acids by the pelleting process (Mohamed et al., 1988). The omega 6:omega 3 ratio observed in the present study was high (19.4:1 on average) compared with the levels of 4 to 5:1 recommended for human consumption (Sim, 1998; Martin et al., 2006), which can be explained by the high levels of C18:2 n6 in sunflower seeds.

According to Tanaka (2005), the plasma level of LDL is influenced by consumed saturated fatty acids. When a diet has high saturated fat levels, the level of LDL in the bloodstream is also high, whereas a diet with higher amounts of unsaturated fats is lower in LDL. This correlation is due to the presence of polyunsaturated fatty acids that elevate the synthesis of LDL receptors in the cell, decreasing the concentration of LDL in the bloodstream (Marzzoco & Bayardo, 1999). In the present work, the LDL levels averaged 92.5 mg/dL in the blood plasma among the treatments, and although not significant, the levels of total cholesterol and LDL were 86 and 69% lower, respectively, in the PSL treatment than in the control treatment. These results are in agreement with those of Santos et al. (2008), who added canola seeds extruded with or without lignosulfonate addition to the diet of dairy cows and did not observe significant effects of the treatment on the blood parameters evaluated.

Although no significant effects of the treatments were observed on the plasma urea levels, values slightly above those levels were observed and suggested by Fergunson et al. (1993). According to those authors, the optimal serum urea nitrogen levels should be less than 20 mg/dL to enable good reproductive performance of the herd. According

to Johnson et al. (2002), it is common for cows fed diets containing oilseeds to have increased urea levels in the blood plasma, most likely due to the increased absorption of ruminal nitrogen.

This result helps us understand why there was no effect of the supposed increased RUP diet with the lignosulfonate use on the milk production and composition. Although not presented here, there was most likely an excess of protein in the diet or a lack of energy or synchronization between the degradation rates of carbohydrates and proteins, which may have elevated the urea nitrogen in the milk. Microbial protein production may have been lower than expected.

The lack of effect of the treatments on the firmness and stickiness of the butter can be explained by the polyunsaturated fatty acid levels and monounsaturated fat found in milk, which were similar for all treatments. The oilseed meals used in the diet of dairy cows increase the polyunsaturated fatty acid level in the milk fat (Bobe et al., 2003), and thus, according to Gonzalez et al. (2003), there may be changes in the physicochemical properties of butter.

Middaugh et al. (1988) tested the use of diets based on sunflower seeds fed to Holstein cows and found that seed addition in the diets caused an increase in the unsaturated fatty acid content in milk fat and, consequently, a higher amount of these fatty acids in the butter, making it softer compared with the control.

Bayourthe et al. (2000) evaluated the effects of supplying unsaturated fatty acids in the diet of dairy cows on the milk fatty acid prolife and the butter physical properties by providing a control diet and diets with canola seeds, crushed canola, extruded canola or canola with the addition of calcium salts. Those authors observed that the treatments containing canola showed higher amounts of unsaturated acids in the milk, resulting in improved butter spreadability.

## Conclusions

Pelleted concentrates containing sunflower seeds can be used for producing milk with a lower fat content in Holstein cows. However, the pelleting process and/or lignosulfonate addition are not effective in protecting the unsaturated fatty acids of the diet or improving the milk fatty acid and butter texture.

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