







Yield, composition, and fatty acid profile of milk from Anglo Nubian goats fed a diet supplemented with vegetable oils

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ABSTRACT - We aimed to evaluate the inclusion of three sources of vegetable oil in the diet of lactating goats on production in 120 days of lactation and the effect of these sources and lactation stage on fortnightly composition and fatty acid profile of goat milk at 20, 50, 80, and 110 days of lactation. A completely randomized design was adopted and 32 Anglo Nubian goats were used, distributed in four treatments: control diet and diets with inclusion of 30 g/kg of dry matter of diet of canola, sunflower, or soybean oil. The dairy production was 182.75 kg, and there was no difference for treatments. Among the constituents, only urea nitrogen was influenced by treatment and presented lower content for control treatment. Day of lactation had an effect on lactose. Defatted dry extract and somatic cell count had a quadratic effect with minimum values around 100 and 33 days of lactation, respectively. The content of urea nitrogen, also with a quadratic effect, was higher at 93 days of lactation. For protein, there was an interaction between treatments and period and, at the end of lactation, its content was increased. The inclusion of vegetable oils promoted reduction in total saturated fatty acids (SFA) and increased the total content of monounsaturated fatty acids (MUFA) and conjugated linoleic acid. The proportions MUFA:SFA and PUFA:SFA, the atherogenicity and thrombogenicity indexes, and the relation hypocholesterolemic fatty acids:hypercholesterolemic fatty acids improved with oil addition in animal diets. The addition of vegetable oil to diets for lactating goats improve the fatty acid profile with no impairment on milk production and composition, and the milk from early stages of lactation has better nutritional quality of the lipid fraction.

Keywords: canola oil, dairy goat, fatty acids, lactation, ruminant nutrition

1. Introduction

Goat milk is a product composed of proteins of high biological value, essential fatty acids, and mineral and vitamin contents that characterize it as a food of high nutritional value. It also has great importance in infant feeding due to its hypoallergenicity and its high digestibility provided by the smaller fat globules in relation to cow milk (Haenlein, 2004). Such characteristics place goat milk in the category of functional foods, that is, in addition to nourishing, it offers health benefits to those who consume it.

One of the major concerns of the population has been the search for healthy eating as a way to prevent the incidence of diseases associated with modern life such as hypertension, cancer, diabetes,

obesity, and coronary heart disease. To reduce the incidence of cardiovascular disease resulting from the increase in plasma cholesterol levels, it is recommended to replace the saturated (SFA) by polyunsaturated fatty acids (PUFA) in the diet (Alvheim et al., 2013).

To determine the nutritional value of fatty foods, the PUFA:SFA ratio, the atherogenicity (AI) and thrombogenicity (TI) indices, and the hypocholesterolemic:hypercholesterolemic fatty acid ratio (h:H) should be considered. Higher PUFA:SFA and h:H and lower AI and TI denote better quality of the lipid fraction of the food, as these values indicate a reduction in the levels of harmful fatty acids and an increase in the levels of hypocholesterolemic fatty acids, lowering the risk of incidence of cardiovascular diseases in humans.

Ruminant-derived foods have a higher SFA content in their fat due to the ruminal biohydrogenation process undergone by the unsaturated fatty acids (UFA) from the diet provided to these animals (Palmquist and Mattos, 2011). Thus, it is possible to change the lipid profile of ruminant milk fat to make it healthier by increasing the supply of UFA in the diet, which will saturate the enzymatic system of biohydrogenation and allow the "escape" of UFA and biohydrogenation intermediates to be absorbed in the intestine. This strategy can be implemented through the inclusion of vegetable oils in animal feed.

Therefore, the present study was conducted to examine the effect of adding canola (*Brassica napus* L.), sunflower (*Helianthus annuus* L.), and soybean (*Glycine max* L.) oils to the diet of lactating goats on feed intake and milk yield, composition, and fatty acid profile to evaluate the improvements in the quality of goat milk.

2. Material and Methods

The experiment was conducted at an experimental station in Botucatu, São Paulo, Brazil (22°53'08" S and 48°26'42" W, and 837 m above sea level), after approval by the local ethics committee (case no. 29/2012 - CEUA).

Thirty-two primiparous and multiparous Anglo Nubian goats were allocated to four treatments (eight goats/treatment), namely, control diet and diets including 30 g/kg (diet dry matter) of canola, sunflower, or soybean oil.

The diets were formulated according to the recommendations of the National Research Council (NRC, 2007) to meet the requirements of lactating goats with an average weight of 50 kg and a 4% fat milk yield of 2 kg/day. The Small Ruminant Nutrition System computer program based on the Cornell Net Carbohydrate and Protein System structure for sheep (Cannas et al., 2004) was used to determine the nutritional composition of the diets, based on a rumen simulation.

As a form of adaptation, the supplements (Table 1) were given to the animals starting one month before the expected start of kidding. In this phase, the oils were gradually incorporated until reaching the level of 30 g/kg in the DM of the total diet.

The kids were suckled twice daily (at 07.00 and 15.00 h) for 30 days postpartum and once daily (at 07.00 h) from 30 to 60 days postpartum. After the suckling period, the dams were milked at 08.00 and 16.00 h to exhaust the residual milk, and at 06.00 and 18.00 h, after the kids were weaned. In the milking interval, the animals remained on *Panicum maximum* cv. Tobiata pasture under rotational grazing with a fixed stocking rate, for an occupation period of three days and a rest period of 30 days. The pasture area, of approximately 0.6 ha, was divided into 11 paddocks of approximately 500 m² that were equipped with automatic drinkers.

After the grazing period, the animals were gathered, milked once again, and distributed into four stalls, according to the treatments, in a pen with suspended, slatted floors with access to a cement-floored solarium. In the stalls, the animals had water and mineral mixture available *ad libitum*. Part of supplement was provided during the milking sections (0.15 kg/animal/milking) and the remainder after the afternoon milking, totaling 1 kg/animal/day. The oil for each treatment was incorporated into the feed at the time of supply to the animals.

To determine the chemical composition of the concentrate, samples of approximately 200 g of each supply were collected after mixing with the respective vegetable oil. Forage samples were obtained by the simulated-grazing method so that the collected material was as similar as possible to that consumed by the animals (De Vries, 1995). For this step, the goats were accompanied upon entering the paddock and their grazing habit was observed. Based on their behavior, representative samples of the consumed forage were collected manually during the three days of stay in the paddock. All samples were kept frozen until analysis.

After thawing, the samples were dried at 55 °C in a forced-air oven for 72 h, processed through a knife mill with a 1-mm mesh sieve, and packed in plastic bags. The dry matter (DM), mineral matter (MM), crude protein (CP), ether extract (EE), cellulose, and lignin (LIG) contents were determined according to AOAC International (Cunniff, 1995), whereas the neutral (NDF) and acid (ADF) detergent fiber levels were determined following the methodology proposed by Van Soest et al. (1991).

The total digestible nutrients (TDN) content was determined according to Weiss (1999) (eq. 1):

$$\text{TDN} = 0.98 * (100 - \text{NDF} - \text{CP} - \text{MM} - \text{EE} - 1) + 0.93 * \text{CP} + 2.25 * \text{EE} + 0.75 * (\text{NDF} - \text{LIG}) * \left(1 - \left(\frac{\text{LIG}}{\text{NDF}}\right) * 0.667\right) - 7 \quad (1)$$

Forage intake was determined using chromium oxide (Cr_2O_3) associated with the internal marker indigestible neutral detergent fiber (iNDF).

To estimate the fecal output, chromium oxide (Cr_2O_3) capsules were administered orally. The marker was supplied in the amount of 2.5 g, once daily at 18.00 h for 10 days, to 20 goats (five animals per treatment). The first five days were used to stabilize the concentration of Cr_2O_3 in the feces and the five last to collect feces, at 06.00 and 18.00 h. A composite fecal sample was made for each animal. These samples were dried for 72 h in a forced-air oven at 55 °C and ground through a knife mill with a 1-mm

Table 1 - Dry matter composition (g/kg), ingredients, and forage (*Panicum maximum* cv. Tobiata) chemical composition

Ingredient	Diet								
	Control	Canola	Sunflower	Soybean					
<i>Panicum maximum</i> cv. Tobiata	500	500	500	500					
Corn	215	0	0	0					
Soybean meal	167	192	192	192					
Wheat bran	82	245	245	245					
Limestone	5	5	5	5					
Mineral matter ¹	28	28	28	28					
Dicalcium phosphate	3	0	0	0					
Oil	0	30	30	30					
Ingredient	Chemical composition								
	DM	MM	MP ²	EE	NDF	ADF	Celulose	Lignin	TDN
Corn	801	14	101	49	121	42	34	10	906
Soybean meal	813	63	276	36	200	90	73	8	802
Wheat bran	811	53	146	39	404	132	93	24	767
<i>Panicum maximum</i> cv. Tobiata	256	83	123	11	679	348	297	20	646
Concentrate									
Control	869	94	171	28	216	88	62	8	773
Canola	875	102	200	86	326	130	80	12	805
Sunflower	872	103	200	79	316	122	81	11	801
Soybean	872	101	201	83	335	132	81	11	803

DM - dry matter; MM - mineral matter; MP - metabolizable protein; EE - ether extract; NDF - neutral detergent fiber; ADF - acid detergent fiber; TDN - total digestible nutrients.

¹ Composition: (g/kg): calcium, 200 g; phosphorus, 70 g; fluorine, 700 mg; sodium, 100 g; sulfur, 10 g; magnesium, 5000 mg; cobalt, 25 mg; copper, 440 mg; chromium, 6 mg; iron, 340 mg; iodine, 48 mg; manganese, 1480 mg; selenium, 20 mg; zinc, 3010 mg; vitamin A, 250,000 UI; vitamin D3, 40,000 UI; vitamin E, 350 UI.

² Cornell Net Carbohydrate and Protein System structure for sheep (Cannas et al., 2004).

mesh sieve. The marker content in the feces was determined by colorimetry after the samples were digested in nitric-perchloric acid, in accordance with the adapted methodology of Bremer Neto et al. (2005).

Fecal dry matter output (FDMO) was calculated as the ratio between the amount of marker supplied (AM supplied) and its content in the feces (AM feces) (eq. 2):

$$\text{FDMO (g/day)} = [(\text{AM supplied})/\text{AM feces}] * 100 \quad (2)$$

Indigestible NDF was the internal marker used to estimate voluntary DM intake from the forage, which was calculated using the equation proposed by Detmann et al. (2001) (eq. 3):

$$\text{DMI (kg/day)} = \{[(\text{FDMO} * \text{iNDFf}) - \text{NDFIs}]/\text{iNDFh}\} + \text{DMIS}, \quad (3)$$

in which DMI (kg/day) = dry matter intake; FDMO = fecal dry matter output (kg/day); iNDFf = iNDF content of the feces (kg/kg); NDFIs = NDF intake from concentrate (kg/day); iNDFh = iNDF content in the forage (kg/kg); and DMIS = dry matter intake from concentrated (kg/day).

The profile of the major fatty acids in the oils, forage, and concentrate used in this study was determined (Table 2). These were extracted according to the methodology of Rodríguez-Ruiz et al. (1998).

The following variables were evaluated: milk yield and components (fat, protein, lactose, solids-not-fat, urea nitrogen, and somatic cell count) at 120 days of lactation and milk fatty acid profile at 20, 50, 80, and 110 days of lactation.

To determine milk yield at 120 days, milk control was performed weekly by individually weighing the milk produced in 24 h on a digital scale with 15-kg capacity and 5-g accuracy. During the suckling period (60 days), to allow the milk control, after the goats from each treatment were milked, the milk was supplied to the kids in collective-suckling buckets.

Milk yield during the evaluated lactation period was calculated using the Fleishman formula (eq. 4) (Procapri, 1994):

$$Y = (E_1 * Y_1) + \left[\sum \left(Y_i + \frac{Y_{(i+1)}}{2} \right) * (Y_i - 1) \right] + (E_n * Y_n) + Y_n, \quad (4)$$

in which E_1 = interval in days between the date of kidding and the first milk control, Y_1 = yield at the first control, Y_i = yield at control i , E_i = interval in days between two consecutive controls, Y_n = yield at the last control, and E_n = interval in days between the date of the last control and the end of lactation.

On the milk control days, the body condition score of the dams was also assessed by palpating their lumbar region to check the mobilization of body reserves and assigning values of 0 (excessively thin) to 5 (excessively fat) on a scale of 0.25 units.

During the 120 days of lactation, samples of approximately 30 mL of milk were collected fortnightly, totaling eight collections. Two-thirds of this volume originated from the morning milking and 1/3 from the afternoon milking. These samples were preserved in Bronopol (2-bromo-2-nitropropane-1-3-diol) to determine the components using a Bentley 2000® instrument (Bentley Instruments, Chasca, MI, USA).

Table 2 - Profile of the main fatty acids in forage (*Panicum maximum* cv. Tobiata), oils, and concentrate

Fatty acid (g/kg)	<i>Panicum maximum</i> cv. Tobiata)	Oil			Concentrate			
		Canola	Sunflower	Soybean	Control	Canola	Sunflower	Soybean
C14:0 (miristic)	6.50	0.80	0.80	0.90	0.90	0.90	0.90	1.00
C16:0 (palmitic)	267.90	51.10	64.30	114.70	169.40	96.40	108.80	142.80
C18:0 (stearic)	25.30	23.10	30.40	29.90	28.40	23.00	28.60	26.70
C18:1 (oleic)	15.00	631.30	278.90	254.40	284.50	517.80	256.60	242.00
C18:2 (linoleic)	183.20	183.00	608.90	527.00	479.80	275.10	570.40	527.90
C18:3n6 (γ-linolenic)	0.00	5.50	1.90	2.40	0.00	1.20	1.30	1.70
C18:3n3 (linolenic)	438.90	66.60	1.30	49.20	23.40	58.10	17.00	41.70
Others	56.80	24.50	10.70	13.60	10.30	21.70	13.60	14.00

To determine the milk fatty acid profile, individual samples of the product (80 mL) were collected monthly and frozen until analysis. After thawing at room temperature, the fat was extracted according to the methodology of Hara and Radin (1978) and then methylated following the method described by Christie (1982). Fatty acids were quantified and determined using a gas chromatograph (Focus CG, Finnigan) with a flame ionization detector and a capillary column (CP-Sil 88, Varian; 100-m long × 0.25-μm film thickness). Hydrogen was used as the carrier gas, at a flow rate of 1.8 mL/min. The initial oven temperature program was 70 °C, with a waiting time of 4 min; 175 °C (13 °C/min), with a waiting time of 27 min; 215 °C (4 °C/min), with a waiting time of 9 min; and then an increase of 7 °C/min until reaching 230 °C, where it remained for 5 min, totaling 65 min. The temperatures of the vaporizer and the detector were, respectively, 250 and 300 °C.

A 1-μL aliquot of the esterified extract was injected into the chromatograph, and fatty acids were identified by comparing the retention times of the methyl esters of the samples with fatty acid standards of butter. The fatty acids were quantified by normalizing the methyl ester areas, and the percentages of fatty acids were obtained using Chromquest 4.1 software (Thermo Electron, Italy), with results expressed as a percentage of the area.

The obtained fatty acid profile was used to calculate the SFA, monounsaturated fatty acids (MUFA), PUFA, omega-3 (ω3), omega-6 (ω6), and conjugated linoleic acid (CLA) contents; the MUFA:SFA, PUFA:SFA, and ω6:ω3 ratios; the atherogenicity index (using the formula $AI = [(C12:0 + (4 * C14:0) + C16:0)] / [(MUFA + \omega6 + \omega3)]$); the thrombogenicity index (using the formula $TI = [(C14:0 + C16:0 + C18:0)] / [(0.5 * MUFA) + (0.5 * \omega6) + (3 * \omega3) + (\omega3/\omega6)]$) (both according to Ulbricht and Southgate (1991)), and the hypocholesterolemic:hypercholesterolemic fatty acid ratio (using the formula $h:H = [(C18:1cis9 + C18:2\omega6 + C20:4\omega6 + C18:3\omega3 + C20:5\omega3 + C22:6\omega3)] / [(C14:0 + C16:0)]$), in accordance with Santos-Silva et al. (2002).

The experiment was laid out in a randomized block design (lactation order), and the data were subjected to analysis of variance. For milk yield, model 1 was used, with means compared by Tukey's test:

$$Y_{ij} = \mu + T_i + B_j + e_{ij}, \quad (5)$$

in which Y_{ij} = milk yield of animal in block j and treatment i , μ = constant inherent to the observations, T_i = effect of treatment i ($i = 1$: canola oil, 2: sunflower oil, 3: soybean oil, and 4: control), B_j = effect of block j ($j = 1$: primiparous and 2: multiparous), and e_{ij} = random error referring to observation $Y_{ij} \sim N(0; \sigma^2)$.

The traits pertaining to the chemical composition and fatty acid profile of milk were analyzed as split plots (model 2), with the main plots being the treatments and the subplots the collection days.

Treatment means were compared using Tukey's test. The effect of collection (lactation stage) was studied by polynomial regression, using the "Sequential Regression" procedure, which evaluates the effect of each independent variable added to the analysis model. The chosen model was that which presented regression analysis of variance (F test) and significant model coefficients (T test) and whose independent variable was responsible for most of the explanation (isolated effect) of the full model. For all analyses and tests, the 5% probability level was adopted.

Model 2:

$$Y_{ijk} = \mu + T_i + B_j + a_{ij} + C_k + T * C_{ik} + e_{ijk}, \quad (6)$$

in which Y_{ijk} = value observed at harvest k , in the animal belonging to block j , receiving treatment i ; μ = constant inherent to the observations; T_i = effect of treatment i ($i = 1$: canola oil, 2: sunflower oil, 3: soybean oil, and 4: control); B_j = effect of block j ($j = 1$: primiparous and 2: multiparous); a_{ij} = effect of the animal in block j receiving treatment i (error referring to the plots); C_k = effect of harvest k ($k = 1, 2, 3, 4, 5, 6, 7$, and 8 for chemical composition and $k = 1, 2, 3$, and 4 for milk fatty acid profile); $T * C_{ik}$ = interaction effect between treatment i and harvest k ; and e_{ijk} = residual effect of the subplots, with $e_{ijk} \sim N(0; \sigma^2)$.

The analyses were performed using SAEG software (UFV, 2000).

3. Results

Dry matter intake averaged 1.382 kg/day between the treatment groups (Table 3), with 0.645 kg of it coming from the forage and 0.737 kg from the concentrate.

Body condition score showed an overall mean of 2.5 and was not influenced by lipid supplementation. This variable decreased linearly during the lactation stages, according to the equation $\hat{Y} = 2.7315 - 0.0035x$, which indicates a reduction in body score of 0.0035 points per lactation day and suggests a slight decrease in the body reserves of the animals during the 120 days of lactation.

Milk yield averaged 182.75 kg in the 120 days of lactation (1.523 kg/day). In terms of milk components, the treatments influenced only the urea nitrogen levels (Table 4). Other components such as somatic cell count and lactose, protein, fat, and solids-not-fat contents remained unchanged. Nonetheless, the stage of lactation influenced the levels of lactose, solids-not-fat, somatic cell count, and urea nitrogen (Table 5).

In this study, somatic cell count increased from 827,000/mL at the beginning of lactation to 4,485,400/mL at the end. Milk urea nitrogen levels showed a quadratic behavior as the lactation period progressed, with a peak of approximately 41 mg/dL at 93 days.

Table 3 - Dry matter (DMI) and nutrients intake according to treatments

Variable	Treatment			
	Control	Canola	Sunflower	Soybean
DMI (kg/day)	1.443	1.483	1.298	1.304
(<i>Panicum maximum</i> cv. Tobiata)	0.693	0.715	0.586	0.585
Concentrated	0.750	0.768	0.712	0.719
DMI ¹⁰⁰ (kg/100 kg BW)	2.78	2.99	2.58	2.41
(<i>Panicum maximum</i> cv. Tobiata)	1.34	1.44	1.17	1.08
Concentrated	1.44	1.55	1.41	1.33
DMI ^{0.75} (g/kg ^{0.75})	74.0	77.7	68.7	64.3
(<i>Panicum maximum</i> cv. Tobiata)	35.5	37.4	31.0	28.8
Concentrated	38.5	40.3	37.7	35.5
Metabolizable protein intake (g/day)	220	240	200	220
EE intake (g/day)	29	74	62	66
NDF intake (g/day)	633	735	623	638
ADF intake (g/day)	307	349	290	299
TDN intake (g/day)	1028	1080	949	955

EE - ether extract; NDF - neutral detergent fiber; ADF - acid detergent fiber; TDN - total digestible nutrients.

Table 4 - Milk composition according to treatments

Variable	Treatment				CV (%)
	Control	Canola	Sunflower	Soybean	
Milk production (kg)	198.28	195.47	169.60	175.00	42.28
Fat (g/kg)	36.70	40.10	41.30	43.00	20.97
Protein (g/kg)	33.50	35.0	33.50	37.10	16.92
Lactose (g/kg)	40.70	41.4	42.90	40.40	15.23
Total solids (g/kg)	120.20	125.9	125.80	129.80	7.73
Defatted dry extract (g/kg)	83.50	85.8	85.50	86.90	5.46
Somatic cell count ($\times 10^3$ /mL)	2366.90	2241.5	1818.70	2056.90	19.27
Ureic nitrogen (mg/dL)	31.87	38.37a	35.21ab	39.35a	37.34

CV - coefficient of variation (%).

Averages followed by the same letter in the rows do not differ by the Tukey test ($P > 0.05$).

There was also an interaction effect between treatment and lactation stage for protein content (Table 6), which decreased until approximately 50 days of lactation and then increased with the amount of milk produced. In the treatment with soybean oil inclusion, the milk protein content decreased by 0.0092% per day, whereas the group fed the control treatment did not show a significant regression for this variable.

Fifty-four fatty acids and isomers were identified in the general milk fatty acid profile. Of these, C18:1c9 (oleic), C16:0 (palmitic), and C18:0 (stearic) were the most abundant, corresponding to 22.19, 21.72, and 15.59%, respectively. Saturated fatty acids were influenced by the effects of treatment and lactation stage (Table 7). Among the short-chain fatty acids, capric (C10:0) and lauric (C12:0) acid levels decreased when vegetable oils were included in the goat diets. The highest stearic acid (C18:0) levels were obtained with the treatments with oil inclusion. A negative linear regression coefficient was found for the C4:0 (butyric), C6:0 (caproic), and C8:0 (caprylic) acids, which implies a reduction in their levels with the advance of lactation (Table 7). Conversely, the C14:0 (myristic), C16:0 (palmitic), and C24:0 (lignoceric) acid levels increased linearly with the progress of lactation, resulting in a reduction of milk fat quality, since myristic and palmitic are the main hypercholesterolemic fatty acids.

The total MUFA content of milk increased by 17.65%, on average (Table 8), in the groups that consumed vegetable oils compared with control treatment. Although not all MUFA identified in milk showed higher means in the treatments with oil inclusion, the total MUFA content rose due to the higher levels of C18:1 isomers (oleic acid) observed in these treatments.

Although regression analysis indicated the influence of the lactation stage on MUFA, none of the tested polynomial models showed a significant effect for regression.

Table 5 - Regression equations for milk components as function of the lactation stage

Variable	Regression	R ²
Lactose (g/kg)	$\hat{Y} = 4.8416 - 0.0107x$	0.95
Defatted dry extract (g/kg)	$\hat{Y} = 9.2013 - 0.0199x + 0.0001x^2$ ($Y_{\text{minimum}} = 8.21$ to $x = 99.5$)	0.92
Somatic cells count (log(x))	$(\log \hat{Y}) = 2.2215 + 0.0040x + 0.00006x^2$ ($Y_{\text{minimum}} = 2.02$ to $x = 33.33$)	0.99
Ureic nitrogen (mg/dL)	$\hat{Y} = 19.2107 + 0.4653x - 0.0025x^2$ ($Y_{\text{maximum}} = 40.9$ to $x = 93.1$)	0.84

R² - coefficient of determination.

Table 6 - Protein content regression as a function of the interaction between treatment and lactation stage

Lactation stage (days)	Treatment			
	Control	Canola	Sunflower	Soybean
15	3.39	3.40	3.55	3.45
30	2.85	3.34	3.44	3.42
45	3.06	3.13	3.15	3.31
60	3.48	3.04	3.12	3.37
75	3.63	3.18	3.22	3.83
90	3.39	3.50	3.34	3.75
105	3.57b	4.09ab	3.36b	4.63a
120	3.39b	4.32a	3.66ab	3.93ab
Treatment	Regression			
Control	ns			
Canola	$\hat{Y} = 3.8124 - 0.0277x + 0.0003x^2$ ($Y_{\text{minimum}} = 3.17$ to $x = 46.2$) (R ² = 0.96)			
Sunflower	$\hat{Y} = 3.8256 - 0.0202x + 0.0002x^2$ ($Y_{\text{minimum}} = 3.32$ to $x = 50.5$) (R ² = 0.89)			
Soybean	$\hat{Y} = 3.1091 - 0.0092x$ (R ² = 0.55)			

x - lactation stage (15, 30, 45, 60, 75, 90, 105, and 120 days); ns - analysis of variance of non-significant regression (F>0.05). Averages followed by the same letter in the rows do not differ by the Tukey test (P>0.05).

The diet with sunflower oil provided a higher total PUFA content (Table 9) in the fat of goat milk. The other studied treatments did not differ from control, possibly because they had a slightly lower PUFA content than the diet with sunflower oil and because these acids are associated with the occurrence of biohydrogenation. Of the identified PUFA, only C18:2c9c12 (linoleic acid) and C18:3n3 (linolenic acid) had their levels influenced by the addition of oil to the diets.

In terms of lactation stage, C18:3n6 (γ -linolenic acid) showed an increasing linear regression coefficient, that is, the content of this fatty acid in milk increases during lactation, improving the nutritional quality of milk, as it is an essential fatty acid.

Table 7 - Saturated fatty acids as a function of treatments and lactation stages

Fatty acid	Treatment				CV (%)
	Control	Canola	Sunflower	Soybean	
C4:0 (butiric)	1.90b	1.99ab	2.18a	2.09ab	15.46
Regression		$\hat{Y} = 2.3161 - 0.1105x$ ($R^2 = 0.97$)			
C6:0 (caproic)	2.42	2.19	2.39	2.39	13.23
Regression		$\hat{Y} = 2.5912 - 0.0965x$ ($R^2 = 0.89$)			
C8:0 (caprilic)	3.07	2.69	2.77	2.70	11.92
Regression		$\hat{Y} = 3.1725 - 0.1472x$ ($R^2 = 0.89$)			
C10:0 (capric)	9.47a	7.71b	7.59b	7.29b	15.77
Regression		-			
C12:0 (lauric)	4.82a	3.66b	3.45b	3.16b	23.32
Regression		ns			
C14:0 (miristic)	10.24a	8.40b	7.94b	7.23b	15.49
Regression		$\hat{Y} = 7.2431 + 0.4835x$ ($R^2 = 0.90$)			
C16:0 (palmitic)	24.54a	20.86b	20.49b	20.98b	8.75
Regression		$\hat{Y} = 19.6987 + 0.8071x$ ($R^2 = 0.95$)			
C18:0 (stearic)	12.26b	15.92a	16.53a	17.66a	15.11
Regression		ns			
C23:0 (tricosanoic)	0.014b	0.019ab	0.02a	0.019ab	34.88
Regression		ns			
C24:0 (lignoceric)	0.005	0.02	0.01	0.01	155.0
Regression		$\hat{Y} = -0.0016 + 0.0053x$ ($R^2 = 0.98$)			
Total saturated fatty acid	71.12a	65.74b	65.59b	65.72b	4.21
Regression		ns			

ns - analysis of variance of non-significant regression ($F > 0.05$) for the linear and quadratic effects of fatty acids as a function of the lactation stage; x - lactation stage (20, 50, 80, and 110 days); R^2 - coefficient of determination; CV - coefficient of variation (%). Averages followed by the same letter in the rows do not differ by the Tukey test ($P > 0.05$).

Table 8 - Monounsaturated fatty acids (MUFA) as a function of treatments and lactation stages

Fatty acid	Treatment				CV (%)	Regression
	Control	Canola	Sunflower	Soybean		
C12:1	0.07a	0.05ab	0.04ab	0.039b	44.68	ns
C16:1c9 (palmitoleic)	0.85a	0.69b	0.71ab	0.65b	15.26	ns
C18:1t6.7.8.9 ¹	0.35b	0.53a	0.60a	0.63a	30.04	ns
C18:1t10.11.12 ¹	1.18c	1.74bc	2.44ab	2.75a	27.68	-
C18:1c9 (oleic)	19.80b	24.08a	22.81a	22.05ab	10.31	-
C18:1c13 ¹	0.56b	0.56b	0.58ab	0.69a	28.30	-
C20:1 (eicosenoic)	0.06b	0.13a	0.08b	0.08b	26.20	-
Total MUFA	25.61b	30.78a	29.87a	29.75a	9.47	ns

ns - analysis of variance of non-significant regression ($F > 0.05$) for the linear and quadratic effects of fatty acids as a function of the lactation stage; x - lactation stage (20, 50, 80, and 110 days); CV - coefficient of variation (%).
¹ C18:1 Isomers.

Averages followed by the same letter in the rows do not differ by the Tukey test ($P > 0.05$).

The milk from the animals fed the diets with sunflower and soybean oil showed higher levels of omega-6 (Table 10). Because canola oil has low omega-6 levels in its composition, the diet with its inclusion reflected this characteristic on the generated product. Likewise, as the control diet did not contain any oil, a lower content of this component was detected in the milk from the animals in this treatment group.

The linear regression coefficient was positive for AI and negative for h:H (Table 10).

None of the major SFA and MUFA were affected by the interaction between the factors, but some of the PUFA were influenced by the interaction between treatment and lactation stage (Table 11).

The levels of C18:2c9t11, which is a conjugated linoleic acid, did not differ between the groups fed sunflower and soybean oils and fluctuated between the periods, with higher values found in relation to the control and canola-oil treatments. In the group that consumed soybean oil, the content of this fatty acid increased linearly with the advancement of lactation, thereby improving milk fat quality, since it is a polyunsaturated fatty acid.

The regression coefficient of the CLA levels as a function of the lactation stage was positive for the treatment with soybean oil, indicating an increase in CLA content during lactation, which translates into better nutritional quality of milk in more advanced stages of lactation.

Table 9 - Polyunsaturated fatty acids (PUFA) as a function of treatments and lactation stages

Fatty acid	Treatment				CV (%)
	Control	Canola	Sunflower	Soybean	
C18:2c9c12 (linoleic)	1.74b	1.80b	2.44a	2.39a	17.98
Regression			ns		
C18:3n6 (γ -linolenic)	0.04	0.06	0.05	0.05	32.80
Regression		$\hat{Y} = 0.0379 + 0.0005x$ ($R^2 = 0.90$)			
C18:3n3 (linolenic)	0.28ab	0.34a	0.23b	0.28ab	28.42
Regression			ns		
C22:5 (docosapentaenoic)	0.08	0.08	0.07	0.07	35.51
Regression		$\hat{Y} = 0.0896 - 0.0284x + 0.0078x^2$ ($Y_{\text{minimum}} = 0.06$ to $x = 1.82$) ($R^2 = 0.99$)			
Total PUFA	2.98b	3.19b	4.18a	4.13b	12.41
Regression			ns		

ns - analysis of variance of non-significant regression ($F > 0.05$) for the linear and quadratic effects of fatty acids as a function of the lactation stage; x - lactation stage (20, 50, 80, and 110 days); R^2 - coefficient of determination; CV - coefficient of variation (%).
Averages followed by the same letter in the rows do not differ by the Tukey test ($P > 0.05$).

Table 10 - Averages of omega-3 (ω_3), omega-6 (ω_6), monounsaturated fatty acids:saturated fatty acids (MUFA:SFA), and polyunsaturated fatty acids:saturated fatty acids (PUFA:SFA) ratios, and quality indexes of the lipid fraction of milk as a function of treatments and lactation stages

Variable	Average	Treatment				CV (%)	Regression
		Control	Canola	Sunflower	Soybean		
ω_3	0.39	0.40	0.46	0.32	0.38	21.59	ns
ω_6	2.37	2.02b	2.05b	2.75a	2.65a	17.20	ns
MUFA:SFA	0.44	0.36b	0.47a	0.46a	0.45a	13.56	ns
PUFA:SFA	0.05	0.04b	0.05b	0.06a	0.06a	14.21	ns
AI	1.94	2.59a	1.79b	1.73b	1.63b	21.33	$\hat{Y} = 1.6019 + 0.1335x$ ($R^2 = 0.95$)
TI	2.74	3.15a	2.54b	2.62b	2.65b	12.29	ns
h:H	0.85	0.66b	0.92a	0.93a	0.89a	15.80	$\hat{Y} = 0.9726 - 0.0489x$ ($R^2 = 0.95$)

AI - atherogenicity index; TI - thrombogenicity index; h:H - hypocholesterolemic:hypercholesterolemic fatty acid ratio; ns - analysis of variance of non-significant regression ($F > 0.05$) for the linear and quadratic effects of fatty acids as a function of the lactation stage; x - lactation stage (20, 50, 80, and 110 days); R^2 - coefficient of determination; CV - coefficient of variation (%).
Averages followed by the same letter in the rows do not differ by the Tukey test ($P > 0.05$).

Table 11 - Average of polyunsaturated fatty acids and ratios influenced by the interaction between treatment and lactation stage

Treatment	Lactation stage (days)				Regression
	20	50	80	110	
C18:2c9t11 (CLA)					
Control	0.498b	0.510c	0.548b	0.682bc	0.560
Canola	0.785ab	0.618bc	0.683ab	0.633c	0.680
Sunflower	1.074a	1.140a	1.069a	1.145ab	1.107
Soybean	0.934ab	1.077ab	1.095a	1.282a	$\hat{Y} = 0.8670 + 0.0035x$ ($R^2 = 0.92$)
C20:5 (eicosapentaenoic)					
Control	0.017	0.020a	0.019	0.017ab	0.018
Canola	0.021	0.020a	0.016	0.023a	0.020
Sunflower	0.012	0.008b	0.010	0.009b	0.010
Soybean	0.016	0.010ab	0.012	0.010b	0.012
C22:6 (docosahexaenoic)					
Control	0.015	0.015	0.011	0.031	0.013
Canola	0.015	0.015	0.011	0.031	0.018
Sunflower	0.011	0.012	0.019	0.024	0.017
Soybean	0.013	0.012	0.014	0.017	$\hat{Y} = 0.0145 - 0.0001x + 0.000001x^2$ ($Y_{\text{minimum}} = 0.012$ to $x = 50$) ($R^2 = 0.99$)
$\omega 6:\omega 3$					
Control	8.228b	5.449b	4.427ab	4.038	5.535
Canola	6.862b	5.205b	3.360b	3.727	4.789
Sunflower	12.731a	10.303a	7.464a	7.063	$\hat{Y} = 13.6897 - 0.0661x$ ($R^2 = 0.93$)
Soybean	8.144b	7.377ab	6.643ab	6.857	7.256

x - lactation stage (20, 50, 80, and 110 days), R^2 - coefficient of determination.
Averages followed by the same letter in the columns do not differ by the Tukey test.

Eicosapentaenoic acid (C20:5) levels showed no differences between the treatment groups in the first and third periods. However, in the second period, the control and canola-oil treatment groups showed higher means for this fatty acid than the sunflower oil-fed group. In the last period, only the treatment with canola oil was superior to the diets with sunflower and soybean oils.

There was no difference between the treatment groups at the different lactation stages for docosahexaenoic acid (C22:6). Nonetheless, for this variable, lactation stage influenced only the group receiving soybean oil, in which it decreased until the 50th day and increased thereafter.

The sunflower oil-fed group was the only one to show a higher $\omega 3:\omega 6$ ratio mean than the control treatment in the first three periods. The negative linear regression coefficient of the $\omega 6:\omega 3$ ratio with the advancement of the lactation stage indicates a reduction in this ratio, which would improve the nutritional quality of milk in this respect.

4. Discussion

The start of lactation is a phase when females have a lower body condition score, which is due to the increased nutrient and energy requirements for milk production (Rodrigues et al., 2006) that leads to mobilization of body reserves. In the final stages of lactation, when milk production declines, there is a tendency for body condition to recover, as the energy present in the ingested feed meets the animal requirements. This fact was not observed in the present study, possibly because lactation was evaluated in the first 120 days, and Anglo Nubian goats have an average lactation period of 236 days (Araújo and Eloy, 1998).

Milk yield did not differ between the treatment groups. This result that can be attributed to the similar intakes of DM, energy, and protein between the groups, which met the requirements of the animals uniformly. The fixed amount of concentrate supplied to the goats (1 kg/animal/day) might also have contributed to this result.

The values observed for milk components are within the normal range for the goat species (Park et al., 2007). According to the literature, variations in the milk composition throughout lactation phases are common and are caused by changes in the amount of milk produced, which will result in higher or lower concentrations of components in the generated product (Brendehaug and Abrahamsen, 1986; Greyling et al., 2004; Mestawet et al., 2012).

Mean lactose contents declined with the progression of lactation, as observed in several studies that examined the composition of milk in the different lactation stages (Gomes et al., 2004; Prasad et al., 2005; Park et al., 2007; Mestawet et al., 2012).

The regression equation for solids-not-fat revealed a quadratic response. This variable reflects the changes that occur in lactose and protein levels during lactation.

As for somatic cell count, studies carried out in Brazil have shown that variations in this parameter in goat milk are due to a number of factors, including lactation stage, birth order, time of year, and type of milking (Peixoto et al., 2010), and that somatic cell count is higher in the final stages of lactation (Souza et al., 2009), as was the case in the present study.

The analysis of urea nitrogen is a tool used to monitor the protein levels in the diet (Hof et al., 1997). High milk urea nitrogen levels mean that the animal does not efficiently utilize protein or that there is a protein deficiency. The protein intake data show similarity between the treatments; however, the urea nitrogen values differed and indicate best utilization of the protein by the animals from the control group.

In experiments with goats carried out by Zambom et al. (2007) and Mouro et al. (2002), milk urea nitrogen concentrations ranged from 17.18 g/dL to 36.87 mg/dL, respectively. The values for this variable are not fixed, as they are dependent on DM intake and the composition of forage and concentrate (Kaufmann, 1982; Oltner et al., 1985). In the present study, the urea nitrogen levels responded quadratically as the lactation period progressed, with a maximum value of approximately 41 mg/dL at 93 days, suggesting variations in the efficiency of the utilization of dietary protein throughout lactation.

The milk protein content is a trait that changes during lactation and tends to behave inversely to milk yield, reaching minimum values in the second month of lactation, which coincides with the period of maximum milk production (Rota et al., 1993). This explains the behavior of this milk component in the groups receiving canola and sunflower oils, in which the milk protein content declined up to approximately 50 days of lactation and subsequently increased according to the amount of milk produced.

Total SFA, C10:0 (capric acid), C12:0 (lauric acid), C14:0 (myristic acid), and C16:0 (palmitic acid) contents decreased with the inclusion of vegetable oils, demonstrating a beneficial effect of their use in the diet of lactating goats, given the constant effort to reduce the total SFA content of animal foods and the fact that C14:0 and C16:0 are the main hypercholesterolemic fatty acids.

The decrease in SCFA, in turn, was a consequence of the escape of long-chain fatty acids from ruminal biohydrogenation (Palmquist et al., 1993). These long-chain fatty acids inhibit the synthesis of the acetyl Co-a carboxylase enzyme (responsible for the *de novo* synthesis of fatty acids in the mammary gland) due to their competition with short- and medium-chain fatty acids for esterification.

The highest stearic acid (C18:0) means were found in the milk from the goats fed diets with oil inclusion. However, despite being saturated, Grundy (1989) reported that stearic acid reduces total plasma cholesterol and LDL levels in humans, as part of it is converted to oleic acid (C18:1) when consumed due to the introduction of a double bond between carbon atoms 9 and 10 (Calder, 1998; Teitelbaum and Walker, 2001). The increased concentration of stearic acid in milk may have been a consequence of the extensive ruminal biohydrogenation of the acids C18:1, C18:2, C18:3n3, and C18:3n6 present in the oils used in the diets in this experiment.

According to Maia et al. (2006), Fernandes et al. (2008), and Strzalkowska et al. (2009), oleic acid is the most abundant MUFA in goat milk, as seen in the present study, in which a higher proportion of this fatty acid was found in the milk. A 17.8% higher oleic acid content was found in the milk from the goats fed canola oil in relation to those fed the control diet.

The higher concentration of oleic acid in the milk from the animals fed the diets with oil inclusion is due to the action of the Δ -9 desaturase enzyme in the mammary gland, whose main function is to convert stearic acid (C18:0), produced in the rumen by the biohydrogenation process, to oleic acid (Palmquist and Mattos, 2011). Of the three evaluated oils, canola oil has the highest oleic acid content (Table 2), and the animals that consumed it possibly had a greater intestinal absorption of this fatty acid on top of its production from stearic acid in the mammary gland.

The higher C18:2c9c12 means observed in the groups fed sunflower and soybean oils and the higher C18:3n3 mean in the group fed canola oil can be explained by the greater supply of these fatty acids from the diet, which was provided by their levels in the respective oils (Table 2).

According to Bomfim et al. (2011), the 18-carbon unsaturated fatty acids that enter the rumen are converted to stearic acid (C18:0) by isomerization and hydrogenation reactions. However, these reactions are limited by the enzymes responsible for this process. Thus, if there is a greater supply of these fatty acids in the diet, the biohydrogenation process will be saturated, allowing the escape of unsaturated C18 fatty acids without modifying their structure.

As stated by Costa et al. (2009), ratios or proportions are suggested as a way to evaluate the risk factor of a food for increasing blood cholesterol levels, as high SFA levels are known to induce an increase in plasma cholesterol levels whereas UFA reduce them. In view of these considerations, decreased SFA contents and increased MUFA, PUFA, MUFA:SFA, and PUFA:SFA values are nutritionally desirable when aiming to reduce plasma cholesterol. In this study, the addition of vegetable oils increased the MUFA:SFA ratio in goat milk when compared with the control diet, which was due to the increase in MUFA and reduction of the SFA content of the milk.

The inclusion of sunflower and soybean oils promoted an increase in the PUFA:SFA ratio in milk. For all the oils used, a reduction was observed in total SFA content, but only the diet with sunflower oil increased the PUFA content, which explains the higher PUFA:SFA ratio for this treatment and the lower values in the control and canola-oil treatments. Soybean oil induced an increase in this variable even though there was no difference in the total PUFA, probably because the PUFA concentration of 4.13% in the group fed the diet with addition of soybean oil (Table 8) was not enough to differ from the 4.18% content presented by the group fed sunflower oil. However, it was sufficient to increase the PUFA:SFA acid ratio.

These data demonstrate that the inclusion of canola, sunflower, and soybean oils in the diet of lactating goats improves the fatty acid profile of their milk, as there was a reduction in total SFA and an increase in total UFA, which are compatible characteristics to make a food nutritionally better for consumer health.

The diets including vegetable oils provided a reduction in AI and TI and an increase in h:H. These indices characterize the degree of risk of a diet to human health. Diets with high AI and TI and low h:H values have a greater harmful effect on human health. The present results suggest an improvement in the nutritional quality of milk fat lipids compared with control diet, which is related to the decrease in the concentrations of SFA in the milk from the animals fed diets with oil addition.

The change seen in the indices throughout lactation indicates a reduction in milk quality in terms of preventing coronary heart disease as lactation progressed. This situation is possibly linked to the increase in the myristic and palmitic acid contents of milk during lactation (Table 7), as they are the fatty acids with the greatest hypercholesterolemic effect and, therefore, used to calculate these indices. The body reserve mobilized at the beginning of lactation may have contributed to the modification of these indices. Nonetheless, nothing can be affirmed due to the lack of knowledge of the mobilized amount and its fatty acid composition.

Conjugated linoleic acid is formed in the rumen as a first intermediate in the biohydrogenation of linoleic acid by the linoleic isomerase enzyme, derived from the anaerobic bacterium *Butyrivibrio fibrisolvens*, which isomerizes linoleic acid preferentially to cis-9 and trans-11 forms (Kepler et al., 1966; Parodi, 1977). This fatty acid can also be synthesized in the mammary gland by the action of the Δ -9 desaturase enzyme on vaccenic acid (C18:1 trans11), another intermediate of biohydrogenation. With the greater supply of linoleic acid in the diets that contain the oils in question, more CLA is formed

in the rumen for intestinal absorption and participation in the composition of milk fat, and the same occurs with vaccenic acid for the synthesis of CLA in the mammary gland. The low linoleic acid (C18:2) content of canola oil may be the cause of the lower CLA levels compared with the sunflower and soybean oils, which promoted an approximation of its mean to control treatment.

Few reports are described in the literature on this interaction. However, the few differences found in the milk fatty acid profile in the different stages of lactation are explained as being due to the negative energy balance at the beginning of lactation that mobilizes body fat, which will be part of the milk composition. According to Demeyer and Doreau (1999), 40-50% of the milk fat originates from lipids synthesized in the mammary gland and the remainder from pre-formed fatty acids absorbed from the bloodstream, whereas 10% of circulating fatty acids originate from the mobilization of body lipids.

The balance between omega-6 and omega-3 acids ($\omega_6:\omega_3$) is an important point to be considered when it comes to fat consumption, because, although essential, high concentrations of ω_6 may favor inflammatory processes that lead to arteriosclerosis due to its tendency to increase platelet aggregation (Young et al., 1998). Thus, lower values for this ratio mean an increase in the ω_3 content or a reduction in ω_6 , which improves the quality of dietary fat in terms of consumer health.

5. Conclusions

The inclusion of 30 g of canola, sunflower, or soybean oils per kilogram of dry matter in the diet of lactating goats does not affect feed intake and improves the milk fatty acid profile without influencing its yield or composition. Therefore, it can be an alternative to increase the energy density of the animal diet and to manipulate the fatty acid profile of milk.

The milk produced in the early stages of lactation has better quality for the health of consumers due to its lipid-fraction quality indices (atherogenicity, thrombogenicity, and hypocholesterolemic:hypercholesterolemic fatty acid ratio).

Among the studied oils, sunflower oil has more advantages than the others for the fatty acid profile. Nevertheless, soybean oil resembles sunflower oil in several characteristics, leaving the option to use these oils at the discretion of the producer, depending on their cost.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: H.C. Gonçalves. Data curation: E.P. Brito, H.F.B. Gomes and H.C. Gonçalves. Formal analysis: E.P. Brito, P.R.L. Meirelles and H.C. Gonçalves. Funding acquisition: H.C. Gonçalves. Investigation: A.C.T. Chávvari, R.O. Marques, H.F.B. Gomes and R.V. Lourençon. Methodology: A.C.T. Chávvari, R.O. Marques and H.C. Gonçalves. Project administration: A.C.T. Chávvari. Supervision: G.I.L. Cañizares and H.C. Gonçalves. Validation: R.V. Lourençon. Visualization: G.I.L. Cañizares and P.R.L. Meirelles. Writing-original draft: A.C.T. Chávvari. Writing-review & editing: H.F.B. Gomes.

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