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Fatty acid profiles of milk and *Minas frescal* cheese from lactating grazed cows supplemented with peanut cake

Weiler Giacomazza Cerutti[1], Julio Viegas[1], Analívia Martins Barbosa[2], Ronaldo Lopes Oliveira[2]*, Carina Anunciação Dias[3], Emellinne Souza Costa[2], José Laerte Nornberg[1], Gleidson Giordano Pinto de Carvalho[2], Leilson Rocha Bezerra[4] and Alisson Minozzo Silveira[1]

[1] Departmento de Zootecnia, University of Bahia Santa Maria, Brazil
[2] School of Veterinary and Animal Science, Federal University of Bahia, Salvador, Bahia 40170110, Brazil
[3] Department of Animal Science, Federal University of Reconcavo Baiano, Cruz das Almas, Bahia 44380000, Brazil
[4] Department of Animal Science, Federal University of Piaui, Bom Jesus, Piaui 6490000, Brazil

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Milk and *Minas frescal* cheese were evaluated from crossbred Holstein × Gir cows that were fed diets enriched with 0, 33, 66 and 100% inclusion levels of palm kernel cake in a concentrated supplement in replace of soybean meal. Eight crossbred lactating cows were distributed (four animals × four treatments × four periods) in the experimental design of double 4 × 4 Latin squares. The capric (C : 10, $P = 0{\cdot}0270$), undecylic (C : 11, $P = 0{\cdot}0134$), and lauric (C : 12, $P = 0{\cdot}0342$) saturated fatty acid concentrations and CLA (C18 : 2c9t11, $P = 0{\cdot}0164$) of the milk fat decreased linearly with an increasing percentage of peanut cake in the diet. The increased peanut cake content (100%) in the diet was associated with a linear decrease in C : 10 ($P = 0{\cdot}0447$), C : 12 ($P = 0{\cdot}0002$), mirystic (C : 14, $P < 0{\cdot}0001$) and palmitic (C : 16, $P < 0{\cdot}0001$) saturated fatty acid concentrations and an increase in arachidic, lignoceric, palmitoleic and elaidic acid levels in the *Minas frescal* cheese fat made from the milk. Both the milk and the *Minas frescal* cheese showed a linear decrease in the concentration of monosaturated fatty acids ($P < 0{\cdot}0001$), atherogenicity index, and thrombogenicity index ($P < 0{\cdot}05$), while the hypocholesterolaemic: hypercholesterolaemic and omega 6: omega 3 ($P > 0{\cdot}05$) ratios were not influenced by the different peanut cake levels. The inclusion of up to 100% peanut cake as a substitution for soybean meal in the concentrate of grazing lactating cows resulted in changes in the nutritional quality of their milk products, as indicated by the increase in polyunsaturated fatty acids and the decrease of saturated fatty acids (lauric, myristic, and palmitic).

Keywords: Fatty acids, lipid profile, oilseed cake, omega 3.

The fat of milk and dairy products has long been associated with a variety of human diseases due to its high content of saturated fatty acids. However, recent studies have revealed that some components of milk fat, such as conjugated linoleic acid (CLA), are beneficial to human health (Oliveira et al. 2015a). A demand for milk and dairy products with a healthier fatty acid profile and/or more functional or nutraceutical properties is booming in developing countries, which has prompted researchers worldwide to evaluate different methods for increasing the level of polyunsaturated fatty acids in the milk and dairy products of various ruminant species.

Dairy cow nutrition is instrumental in determining the quality of milk constituents and milk derivatives. The nature of the feed ingested by ruminants is one of the factors that affect the quality of both dairy products and meat. By-products from the biofuel industry are among the types of food sources that are lower in cost and have potential use in ruminant feed (Oliveira et al. 2015b). Furthermore, the inclusion of cakes from palm fruits in ruminant diets has attracted the attention of the scientific community and stimulated research (Costa et al. 2015; Silva et al. 2015) on the influence of these products on the production and quality of milk and cheese (Oliveira et al. 2015b). Peanut cake is a by-product of the extraction of peanut seed oil and has a high nutritional value, particularly in terms of the levels of protein (41–45%) and lipids (8–9%)

*For correspondence; e-mail: ronaldooliveira@ufba.edu.br

(Abdalla et al. 2008; Oliveira et al. 2013; Gonzaga Neto et al. 2015). However, these parameters can vary in quality, depending on the method used to obtain the oil (Correia et al. 2011).

The use of agro-industrial by-products can be an important tool for reducing production costs and increasing productivity (Oliveira et al. 2011; Silva et al. 2012; Gonzaga Neto et al. 2015). Therefore, it is important to determine the optimal amount of peanut cake to include in the diet of lactating cows. It has also been hypothesised that the use of peanut cake in the diet of lactating cows can improve the fatty acid profile of the milk and *Minas frescal* cheese produced. Thus, the objective of this study was to evaluate the fatty acid profiles of the milk and *Minas frescal* cheese from grazed crossbred cows fed diets supplemented with peanut cake in replacement of soybean meal.

Material and methods

Ethical considerations

This study was carried out in strict accordance with the recommendations of the Guide for the National Council for Animal Experiments Control (NCAEC). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Federal University of Bahia, Salvador, BA, Brazil (Permit Number: 17–2014).

Experimental design, animals, and feeding

The experiment was conducted at the Experimental Farm of the School of Veterinary Medicine at the Federal University of Bahia, located in the municipality of São Gonçalo dos Campos in the state of Bahia (BA). The farm is located in the mesoregion of north-central Bahia and the microregion of Feira de Santana (BA) at 12°23′58″S and 38°52′44″W.

Eight Holstein × Gir crossbred cows were used in the experiment in a double 4 × 4 Latin square. The cows had a mean weight of 507 kg (±35), exhibited multiparous lactation (three lactations) and were between their 45th and 90th days of lactation. Ten days before the first experimental period (described below), the experimental herd was dewormed (1% ivermectin) and supplemented with an injectable solution containing vitamins A, D, and E (Adethor, Tortuga®, Brazil) together with a mixture of mineral salts, B complex vitamins, and amino acids (Mod Plus). The experiment lasted 60 d and was divided into four periods of 15 d, with each period consisting of 11 d of adaptation and 4 d of sample collection.

Four experimental supplements were used, with the level of soybean meal being replaced by peanut cake at 0, 33, 66, and 100%. The roughage in the cows' diets was obtained through grazing an 8-ha pasture of *Panicum maximum* cv. Tanzania split into 10 paddocks of 0·8 ha each and enclosed by an electric fence. The pasture was managed using rotational grazing with 3 d of occupation and 27 d of rest, according to a mean forage supply of 10% of the body weight in dry matter, using a put and take system in which the stocking rate was used to maintain the desired supply. All of the cows grazed the same paddocks and had shaded areas and an ad libitum supply of water and mineral supplements to guarantee that differences in FA precursor intake were caused only by the experimental supplements.

The food used as ingredients in the concentrated supplement were corn meal, soybean meal, a vitamin and mineral mixture, and peanut cake at the levels described above (Table 1). The diets were formulated according to the requirements provided by the National Research Council (NRC, 2001) to meet the potential production of 20 kg milk/d with 3·5% fat. Each animal received 3 kg of the supplement divided into two daily meals at 6:00 am and 4:00 pm. These feeding times coincided with the beginning of daily milkings, which were performed mechanically.

Samples of feeds and feces were pre-dried at 55 °C for 72 h, ground using a Willey mill (Tecnal, Piracicaba, São Paulo, Brazil) with a 1 mm sieve, stored in air-tight plastic containers (ASS, Ribeirão Preto, São Paulo, Brazil), and sealed properly until the chemical composition analysis according to the AOAC (1990): levels of the dry matter (DM) (Method 967–AOAC, 1990), ash (Method 942–AOAC, 1990), crude protein (CP) (Method 981.10–AOAC, 1990), and ether extract (EE) (Method 920–AOAC, 1990). To determine the neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents, the methodology of Van Soest et al. (1991) was used with the modifications that were proposed in the Ankom device manual (Ankom Technology Corporation, Macedon, NY, USA). Acid detergent lignin (ADL) was determined according to method 973.18 of AOAC (2002), in which the ADF residue was treated with 72% sulfuric acid. The percentage of total carbohydrates (TC) was assessed using the equation provided by Sniffen et al. (1992): TC (% DM) = 100 − (%MM + %CP + %EE), and non-fibre carbohydrates were determined according to Detmann et al. (2012): NFC = 100 − (%MM + %CP + %EE) − %NDF (Table 1).

Milk sampling and manufacture of Minas frescal *cheese*

The animals were milked with a milking machine twice daily (at 6 and 16 h); during these times, the dietary supplement was provided. Milk samples were obtained between 11 and 15 d of milking (milking × 2).

Milk samples were mixed in a composite sample and stored in a freezer (−20 °C), pasteurised, and processed at the end of each experimental period in the Food Technology Laboratory of the city of Feira de Santana, Bahia State, Brazil, in the dairy sector. A slow pasteurisation (65 °C 30 min^{-1}) method was used, and its efficiency was evaluated using alkaline phosphatase and peroxidase tests with the aid of a Laborclin kit®.

The production of *Minas frescal* cheese followed the flowchart suggested by Silva (2005), and the samples were analysed for their fatty acid profile in the Integrated Center for Laboratory Analysis Development (Núcleo Integrado de Desenvolvimento em Análises Laboratoriais – NIDAL) of the

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Table 1. Proportion of ingredients and chemical composition of the concentrates used to supplement lactating cows in grazing

	Peanut cake levels			
Proportion of ingredients (g/kg)	0%	33%	66%	100%
Corn meal	656·9	651·6	646·4	641·0
Soybean meal	320·5	214·7	108·9	
Peanut cake		111·1	222·2	336·5
Mineral mixture†	22·6	22·6	22·5	22·5
Chemical composition (g/kg DM)‡				
Dry matter§	862·6	865·3	868·1	870·9
Ash	52·6	51·4	50·1	48·8
Crude protein	187·9	186·1	184·3	182·4
Ether extract	37·5	55·6	73·8	92·4
Neutral detergent fibre	135·6	136·7	137·7	138·8
Neutral detergent $fibre_{ap}$ ¶	105·9	106·6	107·3	108·1
Acid detergent fibre	67·8	68·9	70·1	71·3
Acid detergent lignin	12·9	14·7	16·6	18·5
Cellulose	54·9	54·2	53·5	52·8
Hemicellulose	67·8	67·8	67·6	67·5
Total carbohydrates	722·0	706·9	691·8	676·4
Non-fibre carbohydrates	586·4	570·2	554·1	537·6
Fibre carbohydrates	135·6	136·7	137·7	138·8

†Levels in mineral mix: calcium 18 g; phosphorus 81 g; sodium 104 g; magnesium 15·3 g; sulfur 9·6 g; iodine 49 mg; iron 517 mg; selenium 27 mg; cobalt 100 mg; manganese 1·000 mg; fluoride 810 mg; copper 1·600 mg; and zinc 6·000 mg
‡According to Association of Official Analytical Chemists (1995)
§(g/kg) of fresh matter
¶ Neutral detergent fibre corrected ash and protein

Federal University of Santa Maria. In addition, an aliquot (approximately 50 ml) of milk from each animal was collected at the end of each experimental period and frozen (−20 °C) for subsequent analyses at NIDAL.

Milk and Minas frescal *cheese fatty acid analysis*

For each sample, the fatty acid profile, including the isomers of conjugated linoleic acids (CLAs) C18 : 2 cis-9 trans-11 and C18 : 2 trans-10 cis-12 and vaccenic acid (precursor of CLA), was assessed following the extraction of the lipid fraction as reported by Bligh & Dyer (1959). Subsequently, the saponification and esterification of the fatty acids were performed according to Hartman & Lago (1973) to obtain values for fatty acid methyl esters (FAMEs). FAMEs were analysed using gas chromatography in an Agilent 6890 device equipped with a flame ionisation detector (FID) and a fused silica capillary column (SP−2560−100 m × 0·2 mm × 0·2 μm; Supelco). The runs lasted 42 min. The injector temperature was 250 °C, and the detector temperature was 300 °C. The injection was performed in the 'split' mode with a 1 : 100 ratio. The carrier gas was hydrogen with a flow rate of 40 ml/min and 18 psi of pressure at the column head. The peaks of the fatty acids were identified by comparison with the retention times of the standards (CLA: mix of cis-9, trans-11 and cis-10, trans-12 Sigma octadecadienoic acid esters; vaccenic acid: methyl ester of trans 18 : 1 acid from Sigma; other fatty acids: a mix of 37 fatty acid esters from Supelco). The fatty acid profile was expressed as the percentage of the total number of identified fatty acids (Table 2).

The nutritional quality of the lipid fraction was assessed using indices from the analysis of fatty acid composition, calculated as follows: (1) atherogenicity index (AI) = {(C12 : 0 + (4 × C14 : 0) + C16 : 0)}/(∑monounsaturated fatty acids + ∑*n*6 + ∑*n*3) (Ulbrich & Southgate, 1991); (2) thrombogenicity index (TI) = (C14 : 0 + C16 : 0 + C18 : 0)/{(0·5 × ∑ Monounsaturated fatty acids) + (0·5 × ∑*n*6 + (3 × ∑*n*3) + (∑*n*3/∑*n*6)} (Ulbrich & Southgate, 1991); (3) the ratio of hypocholesterolaemic and hypercholesterolaemic fatty acids (h : H = (C18 : 1cis9 + C18 : 2*n*6 + 20 : 4*n*6 + C 18 : 3*n*3 + C20 : 5*n*3 + C22 : 5*n*3 + C22 : 6*n*3)/(C14 : 0 + C16 : 0), according to Santos-Silva (2002). The total saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid contents were also calculated.

Statistical analysis

Lilliefors' and Cochran's and Bartlett's tests were performed on the data to evaluate normality and homogeneity of variance, respectively. Data that met the assumption of normality were analysed using a mixed procedure (Proc Mixed) and regression (REG) procedure in SAS® statistical software (version 9.1.2. Cary, NC, USA) (SAS®, 2014). The significance level was set at a probability of 0·05* and 0·01**.

Results

The levels of C10 : 0 (capric), C11 : 0 (undecanoic), C12 : 0 (lauric), and C18 : 2c9t11 (CLA) fatty acids decreased significantly ($P = 0·0270$, $P = 0·0134$, $P = 0·0342$, and $P = 0·0164$, respectively) with an increasing proportion of peanut cake

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Table 2. Percentage composition of fatty acids in the lipid fraction of grass, concentrate supplements and experimental diets (expressed as % of total identified fatty acids)

				Peanut cake levels				
Fatty acids	Soybean meal	Corn meal	Peanut cake	0%	33%	66%	100%	Tanzania grass
C11 : 0	0·38	0·81	0·04	0·52	1·01	1·50	0·27	1·75
C12 : 0	0·00	0·24	0·00	0·19	0·06	0·00	0·08	0·74
C13 : 0	2·37	2·71	0·77	2·63	3·17	2·27	1·51	5·46
C14 : 0	0·26	0·98	0·07	0·63	0·17	0·42	0·33	1·42
C15 : 0	0·00	0·00	0·03	0·00	0·00	0·00	0·00	0·34
C16 : 0	18·41	17·42	11·30	20·95	18·46	19·03	16·15	23·49
C17 : 0	0·49	0·00	0·16	0·00	0·68	0·69	0·28	1·17
C18 : 0	5·01	4·61	3·26	5·42	4·86	5·47	4·60	5·96
C20 : 0	0·38	0·79	1·66	0·85	2·22	2·28	1·59	0·70
C14 : 1*n*-5	0·00	0·00	0·02	0·00	0·00	0·00	0·00	1·99
C16 : 1*n*-7	0·00	0·40	0·08	0·45	0·33	0·36	0·41	2·72
C18 : 1*n*-9c	16·01	34·62	47·83	34·70	41·96	46·81	47·96	5·23
C18 : 2*n*-6	50·10	36·21	28·51	30·11	21·22	14·62	22·12	16·76
C18 : 3*n*-3	5·77	1·21	0·19	1·78	1·07	0·76	0·67	30·82
C20 : 1*n*-9	0·00	0·00	1·57	0·76	1·25	1·40	0·54	0·00
C20 : 3*n*-3	0·00	0·00	0·06	0·00	0·00	0·00	0·00	0·00
C20 : 5*n*-3	0·82	0·82	4·28	0·00	2·51	3·43	3·50	1·45
C22 : 2	0·00	0·00	0·09	1·02	1·02	0·95	0·00	0·00

in place of soybean meal in the cows' diets. By contrast, the levels of other fatty acids showed no statistically significant differences ($P > 0·05$) between treatments (Table 3).

The concentrations of capric (C10 : 0) ($P = 0·0447$), lauric (C12 : 0) ($P = 0·0002$), myristic (C14 : 0) ($P < 0·0001$) and palmitic (C16 : 0) ($P < 0·0001$) acids of *Minas frescal* cheese decreased linearly with increasing levels of peanut cake as a concentrated supplement (Table 4). Conversely, the levels of saturated arachidic (C20 : 0) ($P < 0·0001$) and lignoceric (C24 : 0) ($P < 0·0001$), and monounsaturated palmitoleic (C16 : 1) ($P < 0·0001$) and elaidic (C18 : 1) ($P = 0·0044$) fatty acids increased linearly with increasing levels of peanut cake. The other groups of fatty acids showed no statistically significant differences ($P > 0·05$) among the treatments.

In general, the FA profiles of the milk and *Minas frescal* cheese were similar. As in the milk, the *Minas frescal* cheese had relatively higher concentrations of saturated fatty acids in the fatty acid profile, namely myristic (10·31%), palmitic (27·76%), and stearic (14·89%) acids. In addition to monounsaturated oleic acid (28·92%), these acids represented 81·88% of the total fatty acids.

The concentration of saturated fatty acids (SFAs) ($P = 0·3329$), the hypocholesterolaemic: hypercholesterolaemic ratio ($P = 0·2211$), omega 3 (*n*3) ($P = 0·2327$) and the ratio of omega 6: omega 3 ($P = 0·0924$) in the milk was not influenced by the different peanut cake levels in the concentrate supplement for cows in grazing (Table 5). However, a linear decrease in AI (atherogenicity index) ($P = 0·0458$), TI (thrombogenicity index) ($P = 0·0166$) and a linear increase of *n*6 (omega 6) ($P = 0·0218$), monounsaturated fatty acids (MUFAs) ($P \leqq 0·0001$) and polyunsaturated fatty acids (PUFAs) ($P = 0·0413$) was observed with increasing levels of peanut cake in the cows' diets. Furthermore, the omega 6, MUFA, and PUFAs concentrations were 9, 13 and 18% higher, respectively, for the highest level of peanut cake (100%) than for the treatment that did not contain the peanut cake supplement (0%).

Regarding the *Minas frescal* cheese, the concentration of polyunsaturated fatty acids (PUFAs) ($P = 0·4763$), omega 6 (*n*6) ($P = 0·1753$) and the omega 6: omega 3 ratio ($P = 0·0924$) of the milk was not influenced by the different peanut cake levels in the concentrate supplement for cows in grazing (Table 5). There was also a linear decrease in AI (atherogenicity index) ($P < 0·0001$), TI (thrombogenicity index) ($P < 0·0001$) and omega 3 (*n*3) ($P = 0·0070$) of the *Minas frescal* cheese from cows supplemented with peanut cake. The cheese also showed a large linear reduction (25%) in the hypocholesterolaemic: hypercholesterolaemic ratio ($P < 0·0001$) and a small reduction (5%) in saturated fatty acids (SFAs) ($P < 0·0001$) for cows given the peanut cake supplement (100%). Finally, there was also a linear increase (10%) in monounsaturated fatty acids (MUFAs) ($P < 0·0001$) with increasing levels of peanut cake in the diet.

Discussion

Ongoing research to evaluate milk as a nutraceutical food by assessing the characteristics of milk and dairy products that contribute to human health is both important and timely. Lower concentrations of saturated MCFA (lauric, myristic, and palmitic) in peanut cake promoted decreases in these fatty acids in the diets that were offered (Table 2) and consequently in the milk and *Minas frescal* cheese that were produced by the cows (Tables 3 and 4).

Milk fat has high concentrations of short chain fatty acids compared with other foods. Therefore, among the medium-

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Table 3. Fatty acid profile (%) of milk fat from grazed cows fed diets supplemented with peanut cake

Component	Peanut cake level (%DM)					
	Acid	0	33	66	100	SEM†	*P* value
C4 : 0	(Butyric)	0·85	0·59	0·60	0·65	0·0576	0·2638
C6 : 0	(Caproic)	0·79	0·53	0·51	0·58	0·0379	0·1746
[1]C8 : 0	(Caprylic)	0·71	0·51	0·49	0·51	0·0276	0·0514
[2]C10 : 0	(Capric)	1·88	1·38	1·32	1·36	0·0762	0·0270
[3]C11 : 0	(Undecanoic)	0·25	0·21	0·19	0·17	0·0124	0·0134
[4]C12 : 0	(Lauric)	2·63	2·00	1·90	1·99	0·1023	0·0342
C14 : 0	(Myristic)	11·27	9·29	9·76	8·89	0·4555	0·0760
C15 : 0	(Pentadecanoic)	1·28	1·24	0·99	1·11	0·0391	0·2137
C16 : 0	(Palmitic)	30·15	27·93	27·77	26·35	0·8442	0·3604
C17 : 0	(Heptadecanoic)	0·87	0·80	0·71	0·80	0·0339	0·6878
C18 : 0	(Stearic)	14·90	16·70	18·08	18·80	0·5992	0·5036
C20 : 0	(Arachidic)	0·28	0·28	0·28	0·38	0·0147	0·5333
C22 : 0	(Docosanoic)	0·08	0·10	0·09	0·05	0·0220	0·3725
C24 : 0	(Lignoceric)	0·10	0·13	0·18	0·17	0·0161	0·3130
C14 :1*n*5	(Myristoleic)	1·01	0·83	0·87	0·85	0·0561	0·2850
C16 : 1*n*7	(Palmitoleic)	1·70	2·08	1·74	1·62	0·0840	0·5099
C18 : 1 (11t)	(Vaccenic)	3·26	3·28	3·86	3·60	0·1556	0·1193
C18 : 1*n*9t	(Elaidic)	0·33	0·34	0·29	0·37	0·0202	0·2411
C18 : 1*n*9c	(Oleic)	24·12	27·97	27·30	28·63	0·8598	0·3956
C20 : 1*n*9	(Eicosenoic)	0·10	0·08	0·11	0·09	0·0167	0·5122
[5]C18 : 2c9t11	(CLA)‡	1·14	1·04	0·80	1·02	0·0522	0·0164
C18 : 2t10c12	(CLA)‡	0·10	0·11	0·11	0·12	0·0072	0·4154
C18 : 2*n*6c	(Linoleic)	1·37	1·41	1·24	1·27	0·4800	0·2169
C18 : 3*n*3	(α-linolenic)	0·50	0·45	0·41	0·39	0·0251	0·7403
C20 : 3*n*6	(Eicosatrienoic)	0·08	0·07	0·09	0·08	0·0177	0·2732
C20 : 4*n*6	(Arachidonic)	0·20	0·23	0·19	0·18	0·0494	0·4231
20 : 5*n*3	(Timnodonic)	0·05	0·05	0·04	0·05	0·0051	0·1852

†Standard error of the mean (SEM)
‡Isomers of conjugated linoleic acid (CLA)

chain fatty acids that were evaluated (myristic acid, C14 : 0; palmitic acid, C16 : 0; stearic acid, C18 : 0; and oleic acid, C18 : 1*n*9), lauric (C12 : 0), myristic (C14 : 0), and palmitic (C16 : 0) saturated fatty acids were predominant and represented an average of 82% of the total fatty acids. However, the addition of peanut cake resulted in a decrease of 0·0048, 0·0006, and 0·005% in the C10 : 0, C11 : 0 and C12 : 0 milk fatty acids, respectively, for each additional 1% of peanut cake included in the concentrate. Therefore, there was a statistically significant decrease in the levels of saturated fatty acids, which are undesirable for human consumption. However, the same effect occurred with the C18 : 2c9t11 (CLA) fatty acid, which showed a decrease of 0·001 for each 1% of soybean meal replaced with peanut cake. Stearic acid, which accounted for 17% of the total fatty acids on average in this study, is a long-chain fatty acid that consists of 18 carbon atoms without double bonds.

Among the medium-chain fatty acids, special attention must be given to saturated fatty acids C16 : 0 (palmitic acid) and C14 : 0 (myristic acid). Myristic acid is the most hypercholesterolaemic and has a four-fold greater potential than palmitic acid to raise the plasma cholesterol concentration (Akbaridoust et al. 2015; Oliveira et al. 2015a). According to Baggio & Bragagnolo (2007), these fatty acids are of concern due to their hypercholesterolaemic properties (they increase levels of bad cholesterol, i.e., low-density lipoprotein or LDL). However, it is important to emphasise that not all saturated fatty acids are considered hypercholesterolaemic.

In this study, myristic acid accounted for only 9·8% of the total fatty acids and showed a decreasing trend ($P < 0·10$) in response to increasing peanut cake levels in the diet. Palmitic acid (C16 : 0), which had a mean proportion of 28·3%, is reported to have the smallest hypercholesterolaemic effect. Therefore, only 38·1% of the total fatty acids observed in the milk fat in this study (Table 3) are considered harmful to human health.

Oleic acid (C18 : 1*n*9) was the unsaturated fatty acid that contributed the most to the overall composition of acids in the milk in *Minas frescal* cheese. According to Palmquist & Jenkins (1980), one possible effect of increased unsaturated fatty acids (UFAs) in ruminant diets is an increased de novo synthesis of milk fat due to reductions in the enzymatic activity of acetyl-CoA carboxylase. This could explain the decrease in hypercholesterolaemic FAs observed with the increasing dietary levels of peanut cake in this study.

From the results of this study, it can be inferred that the peanut cake supplements contributed to the variation in

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Table 4. Fatty acid profile (%) of *Minas frescal* cheese fat from grazed cows fed diets supplemented with peanut cake

		Peanut cake level					
Fatty acids		0%	33%	66%	100%	SEM†	*P*-value
C4 : 0	(Butyric acid)	0·70	0·58	0·76	0·84	0·040	0·6640
C6 : 0	(Caproic acid)	0·76	0·68	0·74	0·79	0·031	0·3375
C8 : 0	(Caprylic acid)	0·70	0·64	0·64	0·64	0·022	0·0662
C10 : 0*	(Capric acid)	1·78	1·64	1·55	1·51	0·051	0·0447
C11 : 0	(Undecanoic acid)	0·25	0·23	0·23	0·22	0·009	0·5547
C12 : 0**	(Lauric acid)	2·57	2·37	2·25	2·12	0·064	0·0002
C14 : 0**	(Myristic acid)	11·03	10·6	10·12	9·48	0·241	<0·0001
C15 : 0	(Pentadecanoic acid)	1·17	1·15	1·15	1·10	0·023	0·0828
C16 : 0**	(Palmitic acid)	29·10	28·2	28·02	25·6	0·663	<0·0001
C17 : 0	(Heptadecanoic acid)	0·84	0·83	0·80	0·72	0·027	0·0787
C18 : 0	(Stearic acid)	16·00	16·9	12·14	14·5	0·645	0·0637
C20 : 0	(Arachidic acid)	0·22	0·27	0·34	0·36	0·030	<0·0001
C24 : 0**	(Lignoceric acid)	0·10	0·12	0·15	0·16	0·215	<0·0001
C14 : 1*n*5	(Myristoleic acid)	1·06	1·01	1·02	0·91	0·039	0·1251
C16 : 1*n*7**	(Palmitoleic acid)	1·84	1·84	1·91	2·00	0·425	<0·0001
C18 : 1 (11t)	(Vaccenic)	2·72	2·76	3·44	3·45	0·222	0·2870
C18 : 1*n*9t**	(Elaidic)	0·33	0·35	0·43	0·50	0·020	0·0044
C18 : 1*n*9c	(Oleic)	25·93	26·9	31·09	31·7	0·787	0·1114
C18 :2c9t11	(CLA)‡	0·96	0·94	0·98	0·86	0·031	0·2751
C18 : 2*n*6c	(Linoleic)	1·06	1·03	1·14	1·03	0·032	0·1797
C18 : 3*n*3	(α-linolenic)	0·43	0·40	0·43	0·37	0·012	0·2102
C20 : 5*n*3	(Timnodonic acid)	0·05	0·04	0·04	0·04	0·002	0·5016

P-value indicates the differences between supplement levels within each feeding strategy *0·05; **0·01
†Standard Error Mean (SEM)
‡Isomers of conjugated linoleic acid (CLA)

Table 5. Profiles of fatty acid groups from milk fat and Minas frescal cheese from grazing cows supplemented with peanut cake

	Peanut cake level (%DM)					
Fatty acid group	0	33	66	100	SEM†	*P*-value
			Milk			
SFA (saturated)	67·80	60·05	61·23	61·92	0·7086	0·3329
MUFA (monounsaturated)**	30·82	32·59	34·24	35·18	0·6166	<0·0001
PUFA (polyunsaturated)*	0·28	0·27	0·31	0·34	0·0941	0·0413
*n*6 (omega 6)*	1·67	1·81	1·81	2·25	0·0807	0·0218
*n*3 (omega 3)	0·59	0·62	0·60	0·73	0·0161	0·2327
*n*6 : *n*3 ratio	2·83	2·91	3·01	3·08	0·0137	0·0924
AI (atherogenicity)*	2·30	1·80	1·80	1·76	0·0832	0·0458
TI (thrombogenicity)*	3·10	2·79	2·76	2·74	0·0812	0·0166
h/H ratio‡	0·63	0·80	0·77	0·86	0·0353	0·2211
			Minas frescal cheese			
SFA (saturated)**	65·43	64·47	59·21	62·11	0·5960	<0·0001
MUFA (monounsaturated)**	31·86	32·95	37·98	35·31	0·5719	<0·0001
PUFA (polyunsaturated)	2·69	2·61	2·79	2·56	0·0614	0·4763
*n*6 (omega 6)	1·20	1·14	1·30	1·19	0·0292	0·1753
*n*3 (omega 3)**	0·48	0·44	0·47	0·41	0·0114	0·0070
*n*6 : *n*3 ratio	2·5	2·6	2·7	2·9	0·1454	0·0921
AI (atherogenicity)**	2·32	2·12	1·83	1·79	0·0572	<0·0001
TI (thrombogenicity)**	3·11	2·97	2·43	2·68	0·0693	<0·0001
h/H ratio**‡	1·19	1·09	0·96	0·90	0·0287	<0·0001

P-value indicates the differences between supplement levels within each feeding strategy *0·05; **0·01
†Standard Error Mean (SEM)
‡Ratio between hypocholesterolaemic and hypercholesterolaemic

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arachidic and lignoceric acids because 50% of medium-chain fatty acids (FAs with 11 to 16 carbon atoms) in milk were produced in the mammary gland by de novo synthesis, i.e., the synthesis of new molecules of fatty acids from precursors absorbed from the blood (Grummer, 1991). Long-chain fatty acids (>17 carbon atoms) also reach the mammary gland through the bloodstream (Demeyer & Doreau, 1999).

The concentration of CLA in milk is related to the amount available for absorption in the small intestine. Therefore, dietary manipulation aimed at increasing ruminal production likely contributes to higher levels of CLA in milk (Pereira et al. 2011; Oliveira et al. 2015a). Increased intake of linoleic acid is the primary means to increase the production of CLA; as such, the use of peanut cake reduced the amount of CLA because peanut cake has a lower linoleic acid concentration (28·5%) compared with soybean meal (50%) (Table 2).

Our results for the CLA concentrations in the fat of *Minas frescal* cheese corroborate those of Dhiman et al. (1999), who noted that the processing of milk into *Minas frescal* cheese does not alter the CLA content. This finding suggests that cheeses containing high concentrations of CLA are produced from milk that also has a high CLA content (Oliveira et al. 2009). *Minas frescal* cheese produced (100 g) with milk from cows that consumed peanut cake (100%) would contain approximately 20 g of fat, 188 mg of CLA, and 124 mg of vaccenic acid. The consumption of this cheese would result in 311 mg of CLA in the human body, considering that 20% of ingested vaccenic acid is transformed into CLA in the body (Palmquist, 2001). Although CLA has been associated with a reduction in the likelihood of developing breast, colon, and uterus cancer (Benninghoff et al. 2015), it is also associated with a reduction in milk fat yield (Hussein et al. 2015), which may impact the producer's profitability.

The ratio of *n*-6 : *n*-3 did not differ among treatments in either the milk ($P = 0{\cdot}0924$) or *Minas frescal* cheese ($P = 0{\cdot}0924$), confirming that the overall mean for the treatments was a ratio below 3 : 1, thus meeting the recommendations of the World Health Organization (WHOQL, 1995), which suggests values below four. The recommended values range from 4 : 1 to 1 : 1 for the ratio of *n*-6 fatty acids to *n*-3 fatty acids because the problem is not the presence of *n*-6 but rather an imbalance of *n*-6 acids with *n*-3 acids. The mean values for the ratio observed in our study were driven by the *n*-6 FA milk levels, which showed a linear increase ($P = 0{\cdot}0218$) with increasing levels of peanut cake in the diet; however, the *n*-3 levels did not change ($P = 0{\cdot}2327$).

Currently, consumers require a high availability of safe and healthy food, and from a food functionality standpoint, there is a demand for foods that have higher nutritional quality (Mills et al. 2011). Increasing the percentage of long-chain, mono- and polyunsaturated fatty acids in milk fat is an interesting approach to produce healthier food because these FAs are linked to a reduction in the incidence of coronary heart disease in conjunction with an increase in high-density cholesterol (HDL) (Parodi, 2016). The AI and TI indicate the potential for stimulated platelet aggregation. The smaller the values of AI and TI, the larger the amount of anti-atherogenic FA in a specific lipid source, and consequently, the greater the potential to prevent the onset of coronary heart disease (Inzucchi et al. 2015). There are currently no recommended values for these indices for dairy products. Therefore, it is suggested that smaller values of these indices indicate a FA profile that is more beneficial for human health.

No statistically significant difference ($P > 0{\cdot}05$) was found among the treatments in the h/H (hypocholesterolaemic : hypercholesterolaemic) ratio of the milk fat. There are no recommended values in the literature for the h/H ratio of dairy products, although a value of 2·0 is used as a reference for meat products (Santos-Silva et al. 2002). There was a reduction in the h/H ratio in *Minas frescal* cheese, and the mean value for the h/H ratio in this study (0·76) was lower than the meat reference value. The h/H ratio is related to the functional activity of FA in the metabolism of lipoproteins for plasma cholesterol transport, and the type and amount of plasma cholesterol transport is related to the risk of cardiovascular disease (Inzucchi et al. 2015).

Taken together, these results suggest that the supplementation of cows' dietary concentrate with peanut cake (100%) in replace of soybean meal is an efficient method for producing milk and cheese fat with a FA profile that is more appropriate for human consumption because it promoted a decrease in the percentage of saturated fatty acids (C10 : 0, C11 : 0 C12 : 0 in milk and C10 : 0, C12 : 0, C14 : 0, C16 : 0 in cheese), the atherogenicity and thrombogenicity indices and in the high concentrations of CLA, all of which contribute to the onset of coronary heart disease.

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