DOI: 10.1590/1808-1657000192015

Quality of Minas fresh cheese made with milk from F1 Holstein/Zebu cows fed diets with different sources of nitrogen compounds

Qualidade do queijo Minas frescal obtido do leite de vacas F1 Holandês/Zebu alimentadas com dietas com diferentes fontes de compostos nitrogenados

Ana Cássia Rodrigues de Aguiar[1], Vicente Ribeiro Rocha Júnior[1]*, Luciana Albuquerque Caldeira[1], José Reinaldo Mendes Ruas[1], Silvio Humberto Cardoso de Almeida Filho[1], Flávio Pinto Monção[1], Marco Túlio Parrela de Melo[1], Paulo Roberto Silveira Pimentel[1]

ABSTRACT: This study aimed to evaluate yield, fatty acid profile, physical, chemical and sensory composition of Minas fresh cheese made with milk from cows fed diets containing different sources of nitrogen compounds (soybean meal, urea, sunflower meal and detoxified castor bean meal). Eight F1 Holstein/Zebu cows with average production of 20 kg milk corrected to 3.5% fat day^{-1} were distributed in two 4 × 4 Latin squares, consisting of four treatments (diets), four animals and four experimental periods. Cheese was produced on the last day of each experimental period. The physical and chemical composition, yield and texture of cheese were similar between diets. The used diets influenced the concentration of fatty acid C11:0, which was higher for diets with soybean meal and sunflower meal; C18:2 C9-T11 (CLA) was higher for diets with urea, soybean meal and sunflower meal and C20:3 for diets with soybean meal and urea. For the other saturated, monounsaturated and polyunsaturated fatty acids, differences were not detected. Different sources of nitrogen compounds in the diet for cows with average production of 20 kg milk corrected to 3.5% fat day^{-1} have no effect on the physical or chemical composition, yield, as well as acceptance of Minas fresh cheese. However, it can influence the fatty acid profile in the cheese fat.

KEYWORDS: sunflower meal; bran castor; soybean meal; urea.

RESUMO: Objetivou-se avaliar rendimento, perfil de ácidos graxos, composição físico-química e sensorial do queijo Minas frescal produzido do leite de vacas alimentadas com dietas com diferentes fontes de compostos nitrogenados (farelo de soja, ureia, farelo de girassol e farelo de mamona detoxicado). Foram utilizadas oito vacas F1 Holandesas/Zebu, com produção média de 20 kg de leite corrigido para 3,5% de gordura dia^{-1}, em dois quadrados latinos 4 × 4, sendo compostos de quatro tratamentos (dietas), quatro animais e quatro períodos experimentais cada. Os queijos foram fabricados no último dia de cada período experimental. A composição físico-química, o rendimento e a textura do queijo foram semelhantes entre dietas experimentais. As dietas utilizadas influenciaram a concentração do ácido graxo C11:0, sendo superior para as dietas com farelo de soja e farelo de girassol; o C18:2 C9-T11 (CLA) mostrou-se superior para as dietas com ureia, farelo de soja e farelo de girassol; e o C20:3 para as dietas com farelo de soja e ureia. Para os demais ácidos graxos saturados, monoinsaturados e poli-insaturados não foram observadas diferenças. Diferentes fontes de compostos nitrogenados na dieta de vacas, com produção média de 20 kg de leite corrigido para 3,5% de gordura, não alteram a composição físico-química, o rendimento nem a aceitação do queijo Minas frescal, entretanto pode influenciar o perfil de ácidos graxos da gordura do queijo.

PALAVRAS-CHAVE: farelo de girassol; farelo de mamona; farelo de soja; ureia.

[1]Departamento de Ciências Agrárias, Universidade Estadual de Montes Claros (UNIMONTES) – Janaúba (MG), Brazil.
*Corresponding author: vicente.rocha@unimontes.br
Received on: 03/12/2015. Accepted on: 03/16/2017

INTRODUCTION

The factors that influence cheese quality include physical and chemical characteristics and composition of the raw material, which, in turn, are greatly influenced by the nutrition of the cow (EIFERT et al., 2006; FERNANDES et al., 2008; NUDDA et al., 2014; SILVA et al., 2007).

Sources of non-protein nitrogen (NPN), such as urea, have a lower cost per unit of nitrogen and are a viable alternative for replacing traditional protein sources. This substitution is possible only because of the ability of rumen microorganisms to convert NPN into high biological value protein (PAIXÃO et al., 2006). However, according to AQUINO et al. (2009), the use of NPN can alter the composition of the milk protein, influencing the industrial processing of the raw material, since the true protein and casein contents have a direct influence on cheese production. In addition, it should be considered that the differences in ruminal degradability of the nitrogen source can influence the metabolizable protein that reaches the duodenum and consequently the availability of amino acids for milk protein synthesis, thus altering the yield for cheese production.

Currently, the addition of cakes and meals from the agroindustry in the feed for dairy cows has influenced the milk composition (LUNA et al., 2008). In this context, with the biofuel policy, we can expect a greater availability of agroindustrial by-products, assuming an economically important role, basically in the form of inputs for animal feed.

According to FERNANDES et al. (2008), Minas fresh cheese is a derivative of milk, which contains more than 20% fat in its composition, and one of the characteristics of bovine milk is the large amount of saturated fatty acids from de novo synthesis that occurs in the mammary gland; these fatty acids have been related to elevated cholesterol levels and the risk of heart disease. In agreement with BENCHAAR et al. (2007), the fatty acid profile of milk can be altered by changes in the ruminal fermentation pattern and ruminal bacteria species. Thus, the use of different sources of nitrogen compounds in the diet for lactating cows could alter the balance of the ruminal microbiota and consequently the fermentation profile and the content that reaches the duodenum. Considering this, VLAEMINCK et al. (2006) stated that part of the fatty acids that make up milk fat comes from the intestinal absorption of membrane lipids from rumen bacteria.

The goal of the present study was to evaluate the yield, the fatty acid profile, and the physical, chemical and sensorial composition of Minas fresh cheese made with milk from F1 Holstein/Zebu cows fed with diets containing different sources of nitrogen compounds.

MATERIAL AND METHODS

The experiment was conducted at the Experimental Farm of the Universidade Estadual de Montes Claros (UNIMONTES), located in the municipality of Janaúba, state of Minas Gerais. Eight F1 Holstein/Zebu cows, with average production of 20 kg milk corrected to 3.5% fat day^{-1}, and with average lactation period at the beginning of the 80-day experiment, were distributed in two 4 × 4 Latin squares, composed of four animals, four treatments and four experimental periods each. Four experimental diets were tested, one for each of the nitrogen sources (soybean meal, urea, sunflower meal, detoxified castor bean meal). The experiment lasted 72 days, divided into four periods of 18 days. The first 14 days of each period were used for the adaptation of the animals to the diets, and the last four days for data collection and sampling.

Animals were kept in a covered shed with metal structure that contained the individual bays of 20 m^2, equipped with troughs and fountains. Diets were formulated according to the NRC (2001) for cows with an average of 500 kg body weight and average production of 20 kg milk corrected to 3.5% fat day^{-1}, to be isoproteic and isoenergetic and supplied to cows twice a day at 8 a.m. and 4 p.m. The forage used was sorghum silage, weighed daily on a digital scale, placed in the respective troughs and mixed with the concentrates of each treatment. The leftovers in the troughs were weighed and recorded daily. Diets were adjusted according to the leftovers, maintaining a 70:30 forage: concentrate ratio, on a dry matter basis, so that the leftovers represented 10% of the amount supplied. The detoxification of castor bean meal was performed according to ANANDAN et al. (2005) using calcium hydroxide.

The chemical composition of ingredients used in the experimental diets was determined according to guidelines described in DETMANN et al. (2012) and the total digestible nutrients (TDN) of the experimental diets was estimated according to the NRC (2001). The proportion of the ingredients and the chemical composition of the diets are listed in Table 1.

Cows were mechanically milked with calves on foot, twice a day, at 6 a.m. and 2 p.m. Samples of milk from each animal were collected twice a day, during the last four days of each period, and a pool of morning and afternoon milk samples was made in proportion to the amount produced in the morning and afternoon. After milking each cow, the milk was homogenized, and a 500 mL sample was collected. Next, these samples were sent to the Laboratory of Animal Products Technology of UNIMONTES — Janaúba *Campus* —, and the physical and chemical analyses were performed on the same day.

In order to determine the physical and chemical characteristics, milk was analyzed, in triplicate, for titratable acidity (ºD) using the phenolphthalein indicator solution (0.1%); pH, with a Tecnopon digital pHmeter, density at 15ºC, by the Quevenne

thermolactodensimeter; percentage fat content by the Gerber method; protein by the Kjeldahl method with multiplication of the nitrogen percentage by the factor 6.38; ash by incineration in muffle at 550ºC; and cryoscopic index (°H), using the LAKTRON 312-L electronic cryoscope. The percentage of lactose was calculated by the difference between the solid constituents (protein, fat and ash). The calculation of total dry extract (TDE) was obtained from the Ackermann disk, and the defatted dry extract (DDE) by the subtraction of the fat content (BRASIL, 2006) (Table 2).

On the last day of each experimental period, Minas fresh cheese was manufactured at the Laboratory of Animal Products Technology of UNIMONTES — Janaúba *Campus*. The milk from each experimental diet was weighed, filtered and subjected to slow pasteurization (65ºC for 30 minutes). After this heat treatment, milk was cooled to 39ºC, at which temperature the calcium chloride (40 mL/100 L) and rennet (30 mL/100 L) were added, diluted in equal parts of filtered water. After a time of 40 to 60 minutes, the milk was coagulated, and the mass was cut with a stainless steel knife into 1.5 to 2 cm cubes, intercalating the stirring and the rest to promote desorption. Cheeses were placed in the plastic molds for drainage of the serum and remained until the turning at 30 minutes, two turns were made, at which point, the mass was salted (700 g/100 L refined white salt) and then cooled at 10–12ºC. On the next day, they were removed from the molds, packed, weighed on a digital scale to determine the yield.

The texture of the cheese samples was determined using a Texturometer — model TAXT from Stabic Micro Systems —, with the aid of a software, directly supplying the cutting force (kg). A Probe Warner Bratzler cell was used.

The gross yield of cheeses was calculated according to the equation of ANDREATTA et al. (2009). The adjusted yield (REAJ) for the moisture content of cheese (Lucey; Kelly, 1994) was calculated considering a value of 57% as reference for the moisture of Minas fresh cheese, according to the equation suggested by OLIVEIRA (1986).

In order to determine the physical and chemical characteristics, cheese was analyzed, in triplicate, for titratable acidity (ºD) using the phenolphthalein indicator solution (0.1%); pH, with a Tecnopon digital pHmeter; percentage fat content by the Gerber method; protein by the Kjeldahl method; fixed mineral residue by elimination of organic matter at a temperature of 550ºC; total solids by evaporation of water from the

Table 1. Proportion of ingredients of the experimental diets (%) and chemical composition of the diets, on a dry matter basis (%).

Ingredients	Experimental diets (% DM)			
	Soybean meal	Urea	Sunflower meal	Detoxified castor bean meal
Sorghum silage	70.00	70.00	70.00	70.00
Soybean meal	11.94	0.00	0.00	0.00
Sunflower meal	0.00	0.00	13.28	0.00
Detoxified castor bean meal	0.00	0.00	0.00	12.24
Ground corn	17.14	27.18	15.80	16.84
Urea: ammonium sulfate (9:1)	0.00	1.90	0.00	0.00
Mineral supplement	0.92	0.92	0.92	0.92
Chemical composition				
Dry matter (%)	30.43	30.78	31.79	30.92
Organic matter (%)	93.18	93.06	93.01	93.27
Crude Protein (%)	12.05	13.06	13.29	12.30
NDIN (%)	0.44	0.41	0.42	0.43
ADIN (%)	0.02	0.02	0.02	0.02
Ether extract (%)	1.15	1.27	2.33	1.73
Total carbohydrates (%)	75.04	76.45	72.61	76.34
Non-fiber carbohydrates (%)	30.5	32.81	27.26	31.78
Neutral detergent fiber (%)	44.54	43.64	45.35	44.56
NDFcp (%)	44.15	40.23	45.32	42.31
Acid detergent fiber (%)	20.6	23.06	21.45	26.43
Lignin	3.02	3.24	3.65	3.14
Total digestible nutrientes*	65.28	65.16	65.43	65.02

NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen; NDFcp: neutral detergent corrected for ash and protein; *NRC (2001).

sample using the oven at 105ºC, moisture was determined by subtraction of the total solids and water activity (Aw) using the Aw meter Aqua Lab model.

After extraction and methylation, the transmethylated samples of fatty acids from the cheese samples were analyzed in a Focus CG-Finnigan gas chromatograph with flame ionization detector, CP-Sil 88 (Varian) capillary column, with 100 m length, 0.25 µm internal diameter and 0.20 µm film thickness. Fatty acids were identified by comparing the retention times of methyl esters of the samples with fatty acid patterns of butter. Fatty acids were quantified by normalizing the areas of methyl esters. Results of the fatty acids were expressed in mg/g fat. The determination of the fatty acid profile was carried out at the Animal Nutrition Laboratory of Escola Superior de Agricultura Luiz de Queiroz (ESALQ) of Universidade de São Paulo (USP) — *Campus* Piracicaba.

The nutritional quality of the lipid fraction was evaluated by the fatty acid composition data, using the calculations for atherogeneity index (AI), as follows:

- (AI) = {(C12:0 + (4 × C14:0) + C16:0)}/(∑MUFA + ∑w6 + ∑w3) and thrombogenicity index (TI) = (C14:0 + C16:0 + C18:0)/{(0,5 × ∑MUFA) + (0,5 × ∑w6 + (3 × ∑w3) + (∑w3 / ∑w6)}, according to ULBRICTH; SOUTHGATE (1991);
- ratio between hypercholesterolemic fatty acids and hypocholesterolemic fatty acids = (C14: 0 + C16: 0)/(monounsaturated + polysaturated) and desirable fatty acids (DFA) = (unsaturated + C18: 0), according to COSTA et al. (2008);
- ratio between polyunsaturated fatty acids and saturated fatty acids and ratio between w6 and w3 (COSTA et al., 2008).

Microbiological analysis of cheeses was carried out at the Laboratory of Animal Products Technology of UNIMONTES — Janaúba *Campus*. In the laboratory, cheese samples were subjected to external cleaning of the packages with 70% alcohol to remove the contaminants, then 25 g of the product was removed aseptically and homogenized in 225 mL 0.1% buffered peptone water to obtain the initial dilution of 10^{-1}, followed by decimal dilutions up to 10^{-3}.

The determination of the most probable number (MPN) of coliforms at 35ºC was performed from dilution 10^{-1} to then transfer 1 mL aliquots into test tubes containing inverted Durham tubes immersed in lauryl tryptose broth. Samples were incubated at 35ºC for 48 hours. In order to confirm the presence of total coliforms, the positive tubes were inoculated in bright green broth. Confirmation of the presence of coliforms at 45ºC was carried out by inoculation in *Escherichia coli* broth from positive tubes in coliform analysis at 35ºC, with incubation at 45ºC for 48 hours. The result was expressed in MPN of total coliforms per gram, according to BRASIL (2003).

Sensory analysis was performed at the Laboratory of Animal Products Technology of UNIMONTES — Janaúba *Campus*. An assessment of cheeses by untrained judges was performed using the sensory acceptance test described by MEILGAARD et al. (1999). The sensory analysis of cheese was performed in four periods, with 30 tasters per period, the samples were coded and cut into cubes weighing 25 g and supplied in disposable cups. Samples with their respective codes were simultaneously served and classified by the tasters to evaluate the overall acceptance, scoring 1 for the least accepted and 9 for the most accepted one. Data were evaluated by calculating the least significant difference (LSD) of the sum of orders of each sample according to the Friedman Method (5% significance level) (MEILGAARD et al., 1999).

As the microorganism population is classified as a discrete quantitative variable, resulting from counting data, data were log transformed [Log (X + 1)], the additivity was tested with the General Linear Models (GLM) procedure through analysis of the predicted values squared, obtaining p=0.7643; normality was tested by the univariate procedure, with the W

Table 2. Physical and chemical composition of milk used for processing Minas fresh cheese, according to different experimental diets.

Variables	Experimental diets			
	Soybean meal	Urea	Sunflower meal	Detoxified castor bean meal
Fat (%)	4.46	4.59	4.61	4.80
Protein (%)	3.34	3.03	3.27	3.06
Lactose (%)	4.60	4.52	4.65	4.58
Ash	0.75	0.76	0.74	0.72
TS (%)	14.11	14.92	13.36	13.89
DDE (%)	8.94	8.66	8.96	8.72
Acidity (ºD)	17	17	17	17
Density (g/mL)	1.029	1.029	1.030	1.029
Cryoscopy (mºH)	-0.530	-0.532	-0.531	-0.532

TS: total solids; DDE: defatted dry extract.

(Shapiro-Wilk) statistics, with p=0.2870 and the homogeneity of variance by the Bartlett's test (p=0.9725).

Data on the physical and chemical composition, fatty acid profile and nutritional indices of the cheese were tested by analysis of variance by the Sisvar software (FERREIRA, 2011), and, when significant, the means were compared by Tukey's test, considering α=0.05.

RESULTS

The results obtained for the physical and chemical characteristics and yield of the Minas fresh cheese (Table 3) were not significantly influenced (p>0.05) by the sources of nitrogen compounds used in the diets for cows.

The analyzed diets had no effect on the total solids or on the concentrations of the fixed mineral residue of the Minas fresh cheese, with mean values of 43.14 and 2.30%, respectively (Table 3). Minas fresh cheese is classified as a high moisture cheese (over 55%) according to current legislation (BRASIL, 2004). Cheeses in the study had a mean moisture content of 56.86%, thus receiving the same classification. The Aw found confirms the high moisture of the cheese, since the mean value for this variable was 0.98. As for titratable acidity and pH, mean values were 0.15% and 6.60, respectively, indicating no effect of the diets on these variables, which may be related to the similarity (p>0.05) of the other physical components of the cheese from the different experimental diets. The texture of cheeses also presented the same behavior, with a mean of 6.46 (N).

The total fatty acids identified and quantified in Minas fresh cheese were 41 (Table 4): 20 saturated fatty acids (SFA); 15 monounsaturated fatty acids (MUFA); and six polyunsaturated fatty acids (PUFA). There were no differences (p<0.05) in the sums of SFA, MUFA and PUFA, presenting mean concentrations of 76.18, 21.20 and 0.65 g/gram of fat, respectively.

The fatty acid profile of Minas fresh cheese showed a significant effect (p<0.05) for undecanoic acid (C11:0) with higher concentrations for diets with soybean meal and sunflower meal, and C18:2 C9-T11 (conjugated linoleic acid – CLA) was higher for diets with urea, soybean meal and sunflower meal, whereas for eicosatrienoic acid (C20:3) the highest concentrations were found for diets with soybean meal and urea. However, for the other saturated fatty acids, as well as for monounsaturated and polyunsaturated fatty acids (Table 4), no significant differences were detected (p>0.05).

As can be observed in Table 4, the fatty acids found in larger proportions in the Minas fresh cheese of cows fed diets containing different sources of nitrogen compounds are myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-c9), whose average percentages were 12.55, 39.63, 10.37 and 14.99 mg/g fat, respectively, in relation to the total fatty acids from cheese fat.

As in the experimental diets, we used the detoxified castor bean meal, originally containing the toxic principles, ricin and ricinin, which can cause serious damages to animal production, it was sought to identify the presence of ricinoleic acid, but it was not observed in cheese produced with milk from cows fed different sources of nitrogen compounds.

As can be seen in Table 5, none of the nutritional evaluation indices of lipid fraction of the Minas fresh cheese was influenced by the different sources of nitrogen compounds evaluated in the diets for cows. The AI and TI values ranged from 5.53 to 6.16 and from 7.37 to 7.61, respectively. The hyper/hypocholesterolemic

Table 3. Physical and chemical composition (%), gross yield (kg/kg) and moisture-adjusted yield (kg/kg) of Minas fresh cheese made with milk from F1 Holstein/Zebu cows fed diets containing different sources of nitrogen compounds, with respective mean values and coefficients of variation (CV).

Variables	Experimental diets				Mean	CV (%)
	Soybean meal	Urea	Sunflower meal	Detoxified castor bean meal		
Fat	17.34[a]	16.71[a]	16.46[a]	15.65[a]	16.54	5.90
Protein	16.68[a]	16.76[a]	16.51[a]	16.62[a]	16.64	4.29
Fixed Mineral Residue	2.26[a]	2.24[a]	2.32[a]	2.36[a]	2.30	10.15
Total solids	42.88[a]	43.48[a]	43.33[a]	42.86[a]	43.14	3.48
Moisture	57.12[a]	56.52[a]	56.67[a]	57.14[a]	56.86	2.64
Gross yield	6.96[a]	6.77[a]	6.42[a]	6.63[a]	6.70	10.15
Adjusted yield	5.98[a]	5.92[a]	5.63[a]	5.74[a]	5.82	10.19
Lactic acid	0.15[a]	0.14[a]	0.14[a]	0.15[a]	0.15	16.41
pH	6.61[a]	6.60[a]	6.61[a]	6.57[a]	6.60	2.03
Texture (N)	7.40[a]	6.20[a]	6.83[a]	5.40[a]	6.46	52.73
Aw	0.98[a]	0.97[a]	0.97[a]	0.98[a]	0.98	1.48

Mean values followed by different letters, in the same row, are significantly different by Tukey's test at 5% significance.

Table 4. Fatty acid profile of the fat of Minas fresh cheese (mg/g fat) made with milk from F1 Holstein/Zebu cows fed diets containing different sources of nitrogen compounds.

Components	Experimental diets				CV (%)	Pr>Fc
	Soybean meal	Urea	Sunflower meal	Detoxified castor bean meal		
Saturated	76.72[a]	75.00a	76.27[a]	76.72[a]	3.64	0.7959
C4:0	2.86[a]	2.37[a]	2.94[a]	3.06[a]	29.13	0.6552
C6:0	2.06[a]	1.55[a]	2.05[a]	2.00a	27.29	0.4750
C8:0	1.49[a]	1.16[a]	1.44[a]	1.23[a]	18.07	0.2100
C10:0	1.76[a]	1.27[a]	1.58[a]	1.73[a]	18.32	0.1237
C11:0	0.07[a]	0.03b	0.05[a]	0.04b	25.64	0.0107
C12:0	3.57[a]	2.74[a]	3.3[a]	2.8[a]	16.63	0.1199
C13:0 ISS0	0.02[a]	0.02[a]	0.02[a]	0.01[a]	22.12	0.6660
C13:0 ANTEIS0	0.08[a]	0.07[a]	0.08[a]	0.07[a]	17.47	0.2137
C13:00	0.15[a]	0.11[a]	0.14[a]	0.12[a]	17.64	0.0855
C14:0 ISS0	0.11[a]	0.14[a]	0.13[a]	0.12[a]	15.90	0.3042
C14:0	13.13[a]	12.32[a]	12.97[a]	11.78[a]	7.10	0.1765
C15:0 ISS0	0.22[a]	0.21[a]	0.21[a]	0.19[a]	12.50	0.3916
C15:0 ANTEIS0	0.33[a]	0.35[a]	0.33[a]	0.33[a]	10.88	0.8894
C15:0	0.22[a]	0.21[a]	0.21[a]	0.19[a]	12.50	0.3916
C16:0 ISS0	0.16[a]	0.22[a]	0.20[a]	0.19[a]	21.35	0.3053
C16:0	39.07[a]	39.44a	38.68[a]	41.3	11.95	0.8678
C17:0 ISS0	0.16[a]	0.16a	0.17[a]	0.12[a]	31.52	0.4660
C17:0	0.50[a]	0.52a	0.47[a]	0.47[a]	17.26	0.8139
C18:0	9.79[a]	11.23a	10.37[a]	10.10[a]	10.04	0.2869
C20:0	0.90[a]	0.75a	0.91[a]	0.60[a]	32.53	0.3140
Monounsaturated	20.42[a]	22.17a	21.07[a]	21.1[a]	11.02	0.7612
C10:1	0.34[a]	0.31a	0.35[a]	0.30[a]	12.94	0.2335
C12:1	0.10[a]	0.08a	0.10[a]	0.08[a]	18.52	0.1767
C14:1 C9	1.10[a]	1.08a	1.15[a]	1.05[a]	8.43	0.5185
C16:1 C9	1.13[a]	1.19a	1.16[a]	1.36[a]	13.35	0.2264
C17:1	0.12[a]	0.12a	0.11[a]	0.13[a]	30.72	0.7532
C18:1 T6-T7	0.23[a]	0.38a	0.34[a]	0.27[a]	32.95	0.3035
C18:1 T10	0.84[a]	0.80a	0.82[a]	0.82[a]	24.45	0.9950
C18:1 C9	14.42[a]	15.73a	14.95[a]	14.86[a]	12.13	0.6864
C18:1 C11	1.15[a]	1.39a	1.09[a]	1.17[a]	15.92	0.2058
C18:1 C12	0.50[a]	0.59a	0.52[a]	0.53[a]	19.50	0.6604
C18:1 C13	0.28[a]	0.31a	0.29[a]	0.32[a]	21.12	0.8490
C18:1 C15	0.12[a]	0.14a	0.12[a]	0.11[a]	48.66	0.9205
C20:1	0.02[a]	0.05a	0.05[a]	0.03[a]	99.17	0.7474
C21:0	0.01[a]	0.01a	0.01[a]	0.1[a]	43.92	0.3623
C22:00	1.6[a]	1.46a	1.55[a]	1.24[a]	20.68	0.3725
Polysaturated	0.67[a]	0.69a	0.61[a]	0.63[a]	42.36	0.2046
C18:2 C9 C12	0.10[a]	0.13a	0.11[a]	0.11[a]	15.00	0.1281
C18:3 n6	0.11[a]	0.11a	0.09[a]	0.08[a]	44.27	0.6684
C18:3 n3	0.31[a]	0.29a	0.29[a]	0.32[a]	22.48	0.9181
C18:2 C9 T11 CLA	0.04[a]	0.04a	0.03[a]	0.02b	23.64	0.0044
C 20:3 n6	0.06[a]	0.07a	0.04b	0.05b	20.29	0.0046
C22:5	0.05[a]	0.05a	0.05[a]	0.05[a]	20.28	0.7336

Mean values followed by different letters, in the same row, are significantly different by Tukey's test at 5% significance.

Table 5. Atherogenicity index (AI), thrombogenicity index (TI), hyper/hypocholesterolemic ratio, desirable fatty acids (DFA) and ratio of polyunsaturated/saturated fatty acids ratio (PUFA/SFA) in Minas fresh cheese (mg/g fat) made with milk from F1 Holstein/Zebu cows fed diets containing different sources of nitrogen compounds, with respective coefficients of variation (CV).

Variables	Experimental diets				CV (%)
	Soybean meal	Urea	Sunflower meal	Detoxified castor bean meal	
AI	6.16[a]	5.60[a]	5.93[a]	5.53[a]	6.75
TI	7.61[a]	7.44[a]	7.40[a]	7.37[a]	12.13
hyper/hypo	2.37[a]	2.27[a]	2.30[a]	2.40[a]	18.04
DFA	30.21[a]	33.41[a]	31.44[a]	31.21[a]	9.56
PUFA/SFA	0.02[a]	0.02[a]	0.02[a]	0.02[a]	20.42
w6/w3	4.38[a]	5.00a	4.57[a]	5.23[a]	20.72

Mean values followed by different letters, in the same row, are significantly different by Tukey's test at 5% significance.

ratio in the Minas fresh cheese had a mean value of 2.34. The ratio of PUFA/SFA had a mean value of 0.2 for Minas fresh cheese. The mean for the desirable fatty acid content was 31.57%. The w6/w3 ratio presented a mean value of 4.8.

The microbiological analysis of the cheese aimed to verify whether the product meets the microbiological requirements set forth by the National Health Surveillance Agency (BRASIL, 2004) for the conduction of sensory analysis. The results showed there was no contamination after pasteurization, that is, in the processing, the ones standard for the group of coliforms at 35ºC is 5.0×10^2 MPN and coliforms at 45°C is 5×10^3 MPN/g (BRASIL, 2004). In diets with soybean meal, urea, sunflower meal and detoxified castor bean meal, the mean coliform populations at 35ºC and coliforms at 45ºC of the Minas fresh cheese were 63×10^1 MPN/g and 3×10^2 MPN/g; 55×10^1 MPN/g and 4×10^2 MPN/g; 8×10^1 MPN/g and 5.6×10^2 MPN/g; and 7×10^1 MPN/g and 6×10^2 MPN/g, respectively. Therefore, the samples were subjected to sensory analysis.

All the scores assigned in the overall acceptance test to the cheeses produced indicated good acceptance by the judges (slightly liked), showing no significant difference ($p>0.05$), regardless of the nitrogen source used in the diet for cows. The mean values of the assigned scores were 6.36, 6.61, 6.35 and 6.21, for the diets containing soybean meal, urea, sunflower meal and detoxified castor bean meal, respectively, with an overall mean of 6.38 and coefficient of variation of 25.41%, thus reaching a score in the hedonic scale closest to the maximum score (9=extremely liked).

DISCUSSION

With respect to the fat content of Minas fresh cheese, the mean value found was 16.54%, justified by the higher content of this component in milk (Table 2) used as raw material for Minas fresh cheese production. Lower fat values, from 13.8 to 14.22%, were verified by AQUINO et al. (2009), who evaluated increasing levels of urea in partial replacement to soybean meal in the diet for cows in the middle of lactation, producing on average 22 kg milk per day. In this case, the lower fat contents of the Minas fresh cheese, in relation to the values observed in the present study, were probably due to the use of milk with lower fat levels, ranging from 2.63 to 2.80%. The high percentage of forage — 70% dry matter supplied in the feed — helps to explain the higher fat contents in the milk and Minas fresh cheese verified in this work.

The mean percentage of crude protein in the cheese was 16.64%, also justified by the protein content in milk. As can be seen in Table 2, the mean percentage of CP in milk, considering the four experimental diets, was 3.18%. According to AQUINO et al. (2009), milk protein values are important, especially casein, since such concentrations directly affect, reducing or increasing, the yield of milk derivatives. Thus, it can be said that the diets evaluated, associated with the level of production and genetics of the animals, provided a good concentration of protein in milk (Table 2), and possibly with no change in casein levels, which may have contributed to the gross and adjusted yields of the cheese produced, with mean values of 6.70 and 5.82 kg/kg, respectively.

AGUIAR et al. (2013) worked with increasing levels of partial replacement of urea with soybean meal for 7/8 Holstein/Zebu cows and reported results similar to the present experiment, with mean total solids and fixed mineral residue of 45.32 and 2.29%, respectively. The results concerning titratable acidity and pH indicate that the cheese produced had satisfactory quality, which must be confirmed by means of a sensory analysis test, which is directly related to the acceptance of the product by the consumer. Moreover, these results characterize the Minas fresh cheese as a light-tasting cheese, with slightly pronounced taste and slight acidity. The values observed for texture indicating that cheese produced with milk from cows fed diets containing different sources of nitrogen compounds may be considered soft.

Considering the modifications observed in the fatty acid profile of the cheese fat, as seen in Table 1, the content of ether extract in the four experimental diets was below 2.5% (dry matter – DM), indicating that this fraction would not be responsible for possible changes in ruminal fermentation profile. One hypothesis for altering the lipid profile of cheese made with milk from cows fed diets containing different sources of nitrogen compounds would be a possible alteration in the balance of rumen microbiota and consequently the fermentation profile and the content that reaches the duodenum.

A probable explanation for a higher proportion of C18:2 C9-T11 (CLA) in cheese made with milk from dairy cows fed diets with soybean meal, urea and sunflower meal would be because polyunsaturated acids are not synthesized by the tissues of ruminants, and their concentration in milk depends strictly on the amounts absorbed by the intestine and the amounts dispensed in the rumen that were not subjected to biohydrogenation (TSIPLAKOU; ZERVAS, 2008). This concentration of CLA reflects the amount that would be available for absorption in the small intestine and it is thus influenced by the manipulation and amount of lipid in the diet. According to WAHLE et al. (2004) and TOOMEY et al. (2006), C18:2 C9-T11 (CLA) is a nutritionally interesting fatty acid for human health. According to BENJAMIN; SPENER (2009), the potential beneficial effects attributed to the cis-9 trans-11 CLA and trans-10 cis-12 CLA isomers are anticarcinogenic, antiatherogenic, antidiabetogenic, immunomodulatory, osteosynthetic and apoptotic activities.

As for the higher concentrations of undecanoic fatty acid (C11:0) in cheese produced with milk from cows fed diets containing soybean meal and sunflower meal, it is assumed that these may have provided a greater availability of precursors for synthesis of short- and medium-chain fatty acids. In agreement with NUDDA et al. (2014), the increase in the proportion of short- and medium-chain FA may be due to the increase of precursors of de novo synthesis, acetate and β-hydroxybutyrate, resulting from ruminal fermentation.

In turn, for C20:3n6 (eicosatrienoic), higher concentrations were observed for cheese produced with diets containing soybean meal and urea, with means of 0.06 and 0.07 mg/g fat, respectively. The concentrations of this acid depend on the intake and degree of ruminal biohydrogenation, mainly due to the variation of the linoleic acid in the diets used.

The highest proportions of the myristic, palmitic, stearic and oleic fatty acids found in cheese fat can be explained by the proportion of these acids in the milk itself, where the saturated ones represent two thirds, and the oleic is the most abundant unsaturated fatty acid in milk (ABREU, 2005), which consequently reflects the fatty acid profile of cheese.

The absence of ricinoleic acid in cheese indicates that the detoxification process with calcium hydroxide, $Ca(OH)_2$, was efficient to eliminate the toxic principles of castor bean meal, being a safe product for animal feed.

For ARRUDA et al. (2012), the indices of atherogenicity and thrombogenicity are used as measures of evaluation and comparison of the quality of different foods and diets. According to TONIAL et al. (2010), AI and TI indicate the potential for stimulating platelet aggregation, i.e., the lower the AI and TI values, the greater the amount of antiatherogenic fatty acid (FA) present in a given oil/fat, and consequently the greater potential of prevention of the onset of coronary diseases.

The hyper/hypocholesterolemic ratio is an index that relates the functional activity of fatty acids in relation to metabolism of plasma cholesterol transport lipoproteins, whose quantification reflects the greater or lesser risk of cardiovascular disease incidence. In the absence of recommended values for this index in dairy products, the reference considered is the value 2 in relation to meat products (SANTOS-SILVA et al., 2002), as the one that expresses the ideal relationship between hypo- and hypercholesterolemic fatty acids. Values inferior to this reference correspond to fats of superior nutritional quality, reflecting the abundance of fatty acids that promote the lowering of the plasma cholesterol (hypocholesterolemic) and thus the reduction of the risk of cardiovascular diseases. According to the DEPARTMENT OF HEALTH AND SOCIAL SECURITY (1984), values below 0.45 for PUFA/SFA have been considered undesirable because of their potential in inducing increased blood cholesterol.

The concentration of DFA is calculated by the sum of the unsaturated fatty acids with stearic acid. Although stearic acid (C18:0) is saturated, its effect is neutral, with lesser implications on the lipid profile, since it can be converted into oleic acid (C18:1) in the organism. In addition, the monounsaturated acids, oleic, and polyunsaturated acids — linolenic and α-linolenic — reduce LDL cholesterol levels and, consequently, the risk of obesity, cancer and cardiovascular diseases in humans (PEREZ et al., 2002).

The w6/w3 ratio is another index commonly used to evaluate the nutritional value of oils and fats. For w6/w3 ratio, desirable values are suggested below 4.0 in the diet for cardiovascular risk prevention (DEPARTMENT OF HEALTH AND SOCIAL SECURITY, 1994). Diets with ratios between 2:1 and 3:1 have been the most recommended because they allow greater conversion of α-linolenic acid into docosahexaenoic acid (DHA, C22:6 *cis*-4 *cis*-7 *cis*-10 *cis*-13 *cis*-16 *cis*-19), reaching maximum values around 2.3:1. In milk fat and derivatives, this ratio tends to be higher, being influenced by the diet, allowing some ruminant products to become important sources of ω-3 FA in the human diet (HAUG et al., 2007). The increase in the ω-6:ω-3 ratio is undesirable from a human health point of view since, although ω-6 FA is considered essential, elevated levels of ω-6 may be responsible for triggering a series of physiological dysfunctions, such as formation of thrombi, atheromas and immunological disorders (MARTIN et al., 2006).

The satisfactory sensory acceptance of the Minas fresh cheese can be explained by the regional tradition of consumption of this product. Therefore, based on these results, it can be considered that the total replacement of soybean meal with alternative sources of nitrogen compounds, such as detoxified castor bean meal, urea and sunflower meal, in the diet for crossbred cows with production of up to 20 kg milk corrected to 3.5% fat day-1, in order to reduce feed costs, does not hinder the acceptance of Minas fresh cheese by consumers.

Therefore, it can be concluded that the use of different sources of nitrogen compounds in the diet for F1 Holstein/Zebu cows, with average production of 20 kg milk corrected to 3.5% fat, has no influence on the physical and chemical composition, yield, as well as the acceptance of Minas fresh cheese. However, it can change the fatty acid profile of cheese fat.

ACKNOWLEDGEMENTS

To Banco do Nordeste/Fundo de Desenvolvimento Científico e Tecnológico (FUNDECI), for financial support; Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for granting scholarships.

REFERENCES

ABREU, L.R. *Leite e derivados:* caracterização físico química, qualidade e legislação. Lavras: UFLA/FAEPE, 2005. 151 p.

AGUIAR, A.C.R.; OLIVEIRA, C.L.; CALDEIRA, L.A.; ROCHA JÚNIOR, V.R.; OLIVEIRA, S.J.; SOARES, C.; SILVA, D.A.; MENEZES, J.C.; BORGES, L.D.A. Consumo, produção e composição do leite e do queijo de vacas alimentadas com níveis crescentes de ureia. *Revista Brasileira de Ciência Veterinária*, v.20, n.1, p.37-42, 2013.

ANANDAN, S.; ANIL KUMAR, G.K.; GHOSH, J.; RAMACHANDRA, K.S. Effect of different physical and chemical treatments on detoxification of ricin in castor cake. *Animal Feed Science and Technology*, v.120, p.159-168, 2005.

ANDREATTA, E.; FERNANDES, A.M.; SANTOS, M.V.; MUSSARELLI, C.; MARQUES, M.C.; GIGANTE, M.L.; OLIVEIRA, A.F. Quality of Minas frescal cheese prepared from milk with different somatic cell counts. *Pesquisa Agropecuária Brasileira*, v.44, p.320-326, 2009.

AQUINO A.A.; PEIXOTO, K.C.; GIGANTE, M.L.; RENNÓ, F.P.; SILVA, L.F.P.; SANTOS, M.V. Efeito de níveis crescentes de uréia na dieta de vacas leiteiras sobre a composição e rendimento de fabricação de queijos minas frescal. *Brazilian Journal Veterinary Research Animal Science*, v.46, n.4, p. 273-279, 2009.

ARRUDA, P.C.L.; PEREIRA, E.S.; PIMENTEL, P.G.; BOMFIM, M.A.D.; MIZUBUTI, I.Y.; RIBEIRO, E.L.A.; FONTENELE, R.M.; REGADAS FILHO, J.G.L. Perfil de ácidos graxos no *Longissimus dorsi* de cordeiros Santa Inês alimentados com diferentes níveis energéticos. *Semina: Ciências Agrárias*, v.33, n.3, p.1229-1240, 2012.

BENCHAAR, C.; PETIT, H.V.; BERTHIAUME, R.; OUELLET, D.R.; CHIQUETTE, J.; CHOUINARD, P.Y. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. *Journal of Dairy Science*, v.90, p.886-897, 2007.

BENJAMIN, S.; SPENER, F. Conjugated linoleic acids as functional food: an insight into their health benefits. *Nutrition & Metabolism*, v.6, 2009.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Métodos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água*. Instrução Normativa n.º 62 de agosto de 2003.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Métodos Analíticos Oficiais Físico-Químicos para Controle de Leite e Produtos Lácteos. Instrução Normativa n.º 22 de 14 de dezembro de 2006. *Diário Oficial da União*, Brasília, 2006. Disponível em: <http://www.agricultura.gov.br>. Acesso em: 10 jan. 2014.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Departamento de Inspeção de Produtos de Origem Animal. Instrução Normativa n.º 4, de 1.º de março de 2004. *Diário Oficial da União*, Brasília, 2004. Disponível em: <http://www.agricultura.gov.br>. Acesso em: 18 fev. 2014.

COSTA, R.G.; MESQUITA, I.V.U.; QUEIROGA, C.R.E.; MEDEIROS, A.N.; CARVALHO, F.F.R.; BELTRÃO FILHO, E.M. Características químicas e sensoriais do leite de cabras Moxotó alimentadas com silagem de maniçoba. *Revista Brasileira de Zootecnia*, v.37, n.4, p.694-702, set. 2008.

DEPARTMENT OF HEALTH AND SOCIAL SECURITY. *Diet and cardiovascular disease*. Report on Health and social subjects, n.28. London: HMSO, 1984.

DEPARTMENT OF HEALTH AND SOCIAL SECURITY. *Nutritional aspects of cardiovascular disease*. Report on Health and social subjects; n. 46. London: HMSO, 1994. 178p.

DETMANN, E.; SOUZA, M.A.; VALADARES FILHO, S.C.; QUEIROZ, A.C.; BERCHIELLI, T.T.; SALIBA, E.O.S.; CABRAL, L.S.; PINA, D.S.; LADEIRA, M.M.; AZEVEDO, J.A.G. *Métodos para análise de alimentos* – INCT – Ciência Animal. Visconde do Rio Branco: Suprema, 2012. 214p.

EIFERT, E.C.; LANA, R.P.; LANNA, D.P.D.; LEOPOLDINO, W.M.; ARCURI, P.B.; LEÃO, M.I.; COTA, M.R.; VALADARES FILHO, S.C. Perfil de ácidos graxos do leite de vacas alimentadas com óleo de soja e monensina no início da lactação. *Revista Brasileira de Zootecnia*, v.35, n.1, p.219-228, 2006.

FERNANDES, M.F.; QUEIROGA, R.C.R.E.; MEDEIROS, A.N.; COSTA, R.G.; BOMFIM, M.A.D.; BRAGA, A.A. Características físico-químicas e perfil lipídico do leite de cabras mestiças Moxotó alimentadas com dietas suplementadas com óleo de semente de algodão ou de girassol. *Revista Brasileira de Zootecnia*, v.37, n.4, p.703-710, 2008.

FERREIRA, D.F. SISVAR: a computer statistical analysis system. *Ciência e Agrotecnologia*, v.35, n.6, p.1039-1042, 2011.

HAUG, A.; HØSTMARK, A.T.; HARSTAD, O.M. Bovine milk in human nutrition – a review. *Lipids in Health and Disease*, v.6, p.25, 2007.

LUCEY, J.; KELLY, J. Cheese yield. *Journal of the Society of Dairy Technology*, v.47, n.1, p.1-14, 1994.

LUNA, P.; BACH, A.; JUARÉZ, M.; LA FUENTE, M.A. Effect of a diet in whole linseed and sunflower oil ongoat milk fatty acid composition and conjugated linoleic acid isomer profile. *Journal of Dairy Science,* v.91, n.1, p.20-28, 2008.

MARTIN, C.A.; ALMEIDA, V.V.; RUIZ, M.R.; VISENTAINER, J.E.L.; MATSHUSHITA, M.; SOUZA, N.E.; VISENTAINER, J.V. Ácidos graxos poliinsaturados ômega-3 e ômega-6: importância e ocorrência em alimentos. *Revista Nutrição*, v.19, p.761-770, 2006.

MEILGAARD, M.; CIVILLE, G.V.; CARR, B.T. *Sensory evaluation techniques*. Boca Raton: CRC Press, 1999. 2 v. 387p.

NATIONAL RESEARCH COUNCIL – NRC. *Nutrient requirements of dairy cattle*. 7. ed. Washington, D.C.: National Academic Press, 2001. 381p.

NUDDA, A.; BATTACONE, G.; BOAVENTURA NETO, O.; CANNAS, A.; FRACESCONI, A.H.D.; ATIZORI, A.S.; PULINA, G. Feeding strategies to design the fatty acid profile of sheep milk and cheese. *Revista Brasileira Zootecnia*, v.43, n.8, p.445-456, 2014.

OLIVEIRA, J.S. *Queijo: fundamentos tecnológicos*. 2. ed. Campinas: Unicamp, 1986. 146p.

PAIXÃO, M.L.; VALADARES FILHO, S.C.; LEÃO, M.I.; VALADARES, R.F.D.; PAULINO, M.F.; MARCONDES, M.I.; FONSECA, M.A.; SILVA, P.A.; PINA, D.S. Ureia em dietas para bovinos: consumo, digestibilidade dos nutrientes, ganho de peso, características de carcaça e produção microbiana. *Revista Brasileira de Zootecnia*, v.35, n.6, p.2451-2460, 2006.

PEREZ, J.R.O.; BRESSAN, M.C.; BRAGAGNOLO, N.; PRADO, O.V.; LEMOS, A.L.S.C.; BONAGURIO, S. Efeito do peso ao abate de cordeiros Santa Inês e Bergamácia sobre o perfil de ácidos graxos, colesterol e propriedades químicas. *Ciência e Tecnologia de Alimentos*, v.22, n.1, p.11-18, 2002.

SANTOS-SILVA, J.; BESSA, R.J.B.; SANTOS-SILVA, F. Effect of genotype, feeding system and slaughter weight on the quality of light lambs: Fatty and composition of meat. *Livestock Production Science*, v.77, p.187-194, 2002.

SILVA, M.M.C.; SILVA, M.M.C.; RODRIGUES, M.T.; BRANCO, R.H.; RODRIGUES, C.A.F.; SARMENTO, J.L.R.; QUEIROZ, A.C.; SILVA, S.P. Suplementação de lipídios em dietas para cabras em lactação: consumo e eficiência de utilização dos nutrientes. *Revista Brasileira de Zootecnia*, v.36, n.1, p.257-267, 2007.

TONIAL, I.B.; OLIVEIRA, D.F.; BRAVO, C.E.C.; SOUZA, N.E.; MATSUSHITA, M.; VISENTAINER, J.V. Caracterização físico-química e perfil lipídico do salmão (*Salmo salar* L.). *Alimentos e Nutrição*, v.21, p.93-98, 2010.

TOOMEY, S.; MCMONAGLE, J.; ROCHE, H.M. Conjugated linoleic acid: a functional nutrient in the different pathophysiological components of the metabolic syndrome? *Current Opinion in Clinical Nutrition & Metabolic Care*, v.9, p.740-747, 2006.

TSIPLAKOU, E.; ZERVAS, G. The effect of dietary inclusion of olive tree leaves and grape marc on the content of conjugated linoleic acid and vaccenic acid in the milk of dairy sheep and goats. *Journal of Dairy Research*, v.75, p.270-278, 2008.

ULBRICHT, T. L. V.; SOUTHGATE, D. A. T. Coronary heart disease: seven dietary factors. *Lancet*, London, v. 338, n. 8773, p.985-992, 1991.

VLAEMINCK, B.; FIEVEZ, V.; TAMMINGA, S.; DEWHURST, R. J.; VAN VUUREN, A.; DE BRABANDER, D.; DEMEYER, D. Milk odd- and branched-chain fatty acids in relation to the rumen fermentation pattern. *Journal of Dairy Science*, v.89, p.3954-3964, 2006.

WAHLE, K.; HEYS, S.; ROTONDO, D. Conjugated linoleic acids: are they beneficial or detrimental to health? *Progress in Lipid Research*, v.43, p.553-587, 2004.