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## Probiotic caprine Coalho cheese naturally enriched in conjugated linoleic acid as a vehicle for *Lactobacillus acidophilus* and beneficial fatty acids

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### ABSTRACT

To obtain a probiotic caprine Coalho cheese naturally enriched in conjugated linoleic acid (CLA), goats' diet was supplemented with soybean oil to produce CLA-enhanced milk, and *Lactobacillus acidophilus* La5 was incorporated into cheeses. CLA concentration and probiotic viability were evaluated during 60 days. Four pilot-scale cheese-making trials were manufactured, in triplicates. Cheeses T1 and T2 were produced with control milk, and T3 and T4 with CLA-enhanced milk. *L. acidophilus* was added to cheeses T2 and T4 during processing. The CLA content (isomer C18:2 *cis*-9, *trans*-11) in T3 and T4 was 246% to 291% higher than in T1 and T2 ( $P < 0.01$ ). Populations of *L. acidophilus* were around  $7.5 \log \text{cfu g}^{-1}$  in T2 and T4 during the study, and the highest CLA content in T4 did not influence the probiotic viability ( $P > 0.01$ ). The CLA-enriched probiotic caprine Coalho cheese obtained is proposed as a vehicle for beneficial microorganisms and fatty acids.

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### 1. Introduction

Ruminant meat, milk, and dairy products are the predominant sources of conjugated linoleic acids (CLA), a group of linoleic acid isomers that have been implicated in the promotion of health and disease risk reduction in humans (Jones et al., 2005). The major CLA isomer in ruminant milk fat, *cis*-9, *trans*-11, was reported to present anticarcinogenic and anti-atherogenic properties in animal experiments, and its concentration in goat milk might be increased with vegetal oil supplementation of animal diets (Bernard, Shingfield, Rouel, Ferlay, & Chilliard, 2009; Matsushita et al., 2007). Nutritional strategies to increase milk CLA content also result in other positive changes in the composition of milk fat, reducing the content of saturated fatty acids and increasing the proportion of monounsaturated and polyunsaturated fatty acids. All these changes in milk fat may be transferred to cheese, since the manufacturing process does not affect the fatty acid composition (Jones et al., 2005; Luna, Bach, Juárez, & de la Fuente, 2008; Luna, de la Fuente, & Juárez, 2005).

As pointed out by Cruz, Buriti, Souza, Faria, and Saad (2009), cheese is one of the most versatile food products, offering

opportunities for many marketing strategies, new technology developments, and for adding health benefits, including the possibility of transforming it into a probiotic food carrier. To develop probiotic cheeses, however, it is necessary to know the effect of all manufacturing steps and inputs on the survival of the added bacteria through the product shelf life. In this context, the use of CLA-enhanced milk might influence the viability of microorganisms in a probiotic cheese, as suggested by studies about the effects of specific fatty acids on lactobacilli growth and properties (Endo, Kamisada, Fujimoto, & Saito, 2006; Kankaanpää, Salminen, Isolauri, & Lee, 2001), and such effects should be evaluated.

Caprine cheese is consumed worldwide and the demand for goat dairy products in Brazil is growing. "Coalho" cheese is a Brazilian partially-cooked or cooked curd cheese, typically produced and widely consumed in the Northeast of Brazil. Its processing technology was adapted for the use of goat milk by researchers of Embrapa Goats and Sheep (Egito & Laguna, 1999), resulting in a product with a distinctive taste.

Due to intrinsic properties as low allergenic potential and high digestibility, besides its nutritional value, goat milk is a food associated with positive health outcomes (Raynal-Ljutovac, Lagriffoul, Paccard, Guillet, & Chilliard, 2008). The improvement of goat milk fatty acid profile and the subsequent supplementation of a caprine cheese with probiotic microorganisms may increase the functional potential of this dairy product, associating the health properties provided by the probiotic strain, the CLA, and the goat milk. The

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present study aimed to evaluate the CLA concentration and the viability of the probiotic strain *Lactobacillus acidophilus* La5 in Coalho cheeses manufactured with goat milk enriched in CLA through dietary manipulation.

## 2. Material and methods

### 2.1. Animal diets and milk production

Milk for cheese manufacture was produced at Goats' Milk Technological Centre of Embrapa Goats and Sheep, Sobral, Ceará State, Brazil. Saanen goats ( $n = 30$ ), with average milk yield of 1.81 kg per day, were divided in two groups, according to animals' age, parturition and individual milk production. Groups were characterized by the diets without and with soybean oil (SBO) for the production of control milk and CLA-enhanced milk, respectively. Diets were formulated to fulfil the nutritional requirements of lactating goats producing daily 2 kg of milk (NRC, 2007), and the proportion of oil added to the goats' diet for increasing CLA content in milk was defined to avoid negative impacts on intake or digestibility, based on a previous trial (unpublished data). For animals of the control milk group, feed was provided as a total mixed ration (TMR), containing hay produced with elephant grass (*Pennisetum purpureum* cv. Napier), ground maize grain, soybean meal and minerals. For the CLA-enhanced milk group, the ground maize grain was partially replaced by ground ear maize and SBO, in the proportion of 3.75% (w/w) of total diet, to achieve an iso-energetic diet. The complete list of ingredients used for formulation of the goats' diets employed in the present study, as well as its respective chemical composition, is shown in Table 1.

Considering the effect of the oil supplementation on milk CLA concentration (Abu Ghazaleh & Holmes, 2007), milk was collected after a 15-day adaptation period to both diets during 18 days, through mechanic milking, twice a day (at 8 a.m. and 2 p.m.). After milking, milk was transported to milk processing facilities, pasteurized (65 °C, 30 min), and immediately refrigerated at 10 °C.

### 2.2. Coalho cheese manufacture

Coalho cheeses were manufactured at the Dairy Processing Pilot Plant of Embrapa Goats and Sheep. Cheeses were produced in pilot

scale (40 L vats) and divided into four trials, denoted T1, T2, T3, and T4. From each vat, bulk milk samples were collected for analysis. Each group of four trials was produced in triplicate, with a break of a one week period between them. Cheeses T1 and T2 were produced with pasteurized milk from goats that received the control diet and the milk from animals with modified diet was employed for production of cheeses T3 and T4. Cheeses T2 and T4 were supplemented with the probiotic culture of *L. acidophilus* La5 (Christian Hansen, Hørsholm, Denmark). All cheeses were manufactured using the mesophilic homofermentative type O lactic culture consisting of *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (R-704; Christian Hansen). All the cultures employed were freeze-dried Direct Vat Set (DVS) cultures.

Milk temperature was adjusted to 35 °C for the addition of calcium chloride (0.1 g L<sup>-1</sup>; Vetec, Rio de Janeiro, Brazil), mesophilic lactic culture (0.01 g L<sup>-1</sup>), probiotic culture (0.1 g L<sup>-1</sup>) and commercial coagulant Ha-la (enzymatic preparation containing liquid protease from *Aspergillus niger* var. *awamori*, activity 1:3000/75 International Milk-Clotting Units – IMCU, Christian Hansen, Valinhos, Brazil, 0.8 mL L<sup>-1</sup>). The amount of probiotic culture added was adjusted, in order to achieve an estimated concentration of at least 7 log cfu mL<sup>-1</sup> in milk, based on the concentration of viable probiotic bacteria in the freeze-dried culture, previously determined.

All vats were kept at 35 °C until forming a curd. The curd was cut cubes (ca. 3 cm<sup>3</sup>) and allowed to drain with gentle agitation. One-half of the whey was removed, heated, and incorporated again to the cheese curd, which achieved a temperature of 42 °C. The cheese curd was maintained at this temperature during 10 min, with intermittent agitation. After removal of 90% (v/v) of whey, the cheese curd was salted with 0.9 g L<sup>-1</sup> NaCl, based on the initial milk volume. The curd was placed in polypropylene cheese basket moulds and pressed with a cheese press (Brasholanda S/A Equipamentos Industriais, Model RP 1000, Serial code P, Curitiba, Brazil, 3.56 N) for 18 h, at 25 °C. Cheeses were removed from the containers and kept at 10 °C for additional 24 h. After that, cheeses were vacuum packed and maintained at 10 °C for up to 60 days.

### 2.3. Compositional analysis

Moisture, ash, fat, protein (% w/w, total matter) were determined for goat milk after pasteurization and for Coalho cheeses on the first day after processing. Chloride content (% w/w, total matter) was also determined for Coalho cheeses. Cheeses of each trial were unpacked, had their rinds removed, were grated, and a representative portion was taken for analysis. Moisture content was determined, in duplicate, through drying 5 g samples at 100–105 °C for 24 h. Ash was determined gravimetrically through heating the dried samples at 550 °C. Fat was determined in triplicate samples, using the Gerber butyrometer method. Chloride content was determined in ashed cheese samples, in triplicates, by titration with silver nitrate. Analytical procedures for moisture, ash, fat and chloride followed the standard methods of the Instituto Adolfo Lutz (IAL, 2008). Protein was estimated by measuring the total nitrogen content in duplicate samples by the micro Kjeldahl method and multiplying by a conversion factor (6.38), according to the AOAC official methods 960.52 and 991.20 (AOAC, 2003).

### 2.4. Fatty acid analysis and estimation of conjugated linoleic acid content in milk and cheeses

Goat milk and Coalho cheeses were analyzed for fatty acids (FA) after 1, 30, and 60 days of ripening, using two batches of each trial. Fat from milk and cheese samples was extracted (Bligh & Dyer, 1959) and FA composition was determined after conversion of FA

**Table 1**  
Ingredients, composition and metabolizable energy of goats' diet.

Parameter	Diets	
	Control – without soybean oil	Modified – with soybean oil
Ingredients (% w/w) <sup>a</sup>		
Hay from elephant grass ( <i>Pennisetum purpureum</i> cv. Napier)	44.37	45.25
Ground maize grain	35.21	–
Ground ear maize	–	34.79
Soybean meal	17.72	13.56
Soybean oil	–	3.75
Dicalcium phosphate	1.23	1.43
Limestone	1.47	1.22
Composition (g 100 g <sup>-1</sup> dry matter)		
Crude protein	14.76	12.39
Neutral detergent fibre	41.79	50.04
Ether extract	2.74	5.97
Calcium	1.09	1.05
Phosphorus	0.48	0.48
Metabolizable energy (Mcal kg <sup>-1</sup> )	2.53	2.43

<sup>a</sup> A dash indicates the ingredient is absent.

into their corresponding methyl esters (Christie, 1982). Fatty acid methyl esters were quantified using a gas chromatograph Shimadzu GC 2010 (Shimadzu, Kyoto, Japan), equipped with a flame-ionization detector (FID) through split injection (1:100) onto a fused silica capillary column (Supelco SP-tm-2560, Supelco, Bellefonte, PA, USA), using a programmed temperature gradient method. N<sub>2</sub>/Air was the carrier gas at a pressure of 212.4 kPa and the injector and detector were at 250 °C. Initial oven temperature was 50 °C. After 86 min, the oven temperature was raised to 220 °C and kept at this temperature for 30 min. The volume of injections was 1.0 µL. Reference standards were used to determine recoveries and correction factors for individual FA (Supelco 37 Component FAME MIX and linoleic conjugated acid methyl ester, Sigma–Aldrich, Bellefonte, PA, USA). Individual FA were identified and quantified by comparing the retention times and areas of their peaks to those observed for their respective standards. Data were collected using the software GC Solution Analysis version 2.30, and expressed as percentage (% w/w) of total FA methyl esters (FAME).

The proportions of CLA (isomer C18:2 *cis*-9, *trans*-11) in whole samples of milk and cheese were estimated according to the Equation (1):

$$\text{CLA (mg 100g}^{-1}\text{)} = \frac{\text{CLAME} \times \left( \frac{\text{CLAME}_{\text{MW}}}{\text{CLAME}_{\text{MW}}} \right) \times \text{FAT} \times 0.933 \times 1000}{\sum \text{FAME} \times \left( \frac{\text{FA}_{\text{MW}}}{\text{FAME}_{\text{MW}}} \right)} \quad (1)$$

where CLAME is the percentage of CLA methyl ester (% w/w, total FAME), CLAME<sub>MW</sub> and CLAME<sub>MW</sub> are the corresponding molecular weights for CLA and CLA methyl ester (280.486 and 294.513, respectively), FAT is the percentage of total fat in samples (% w/w), 0.933 is the coefficient for the mean FA proportion in total milk fat (Glasser, Doreau, Ferlay, & Chilliard, 2007),  $\sum$ FAME is the total sum of individual FA methyl esters (% w/w, total FAME) for which FA<sub>MW</sub> and FAME<sub>MW</sub> are the corresponding FA and FAME individual molecular weights. For this purpose, the percentage of total fat in samples (% w/w) was determined in triplicate milk and cheese samples on the first day after processing through the Gerber butyrometer method, following the analytical procedure of Instituto Adolfo Lutz (IAL, 2008).

### 2.5. Population of *L. acidophilus* in Coalho cheeses

The population of *L. acidophilus* was monitored in Coalho cheeses T2 and T4, in triplicate samples, after 1, 15, 30, 45, and 60 days of ripening. Portions of each cheese sample were collected aseptically (25 g) and blended with 0.1%, w/v, peptone water (225 mL) and serially diluted in the same diluent.

*L. acidophilus* was analyzed by pour-plating 1 mL of each dilution in modified DeMan–Rogosa–Sharpe (MRS) agar, modified by the substitution of glucose for maltose as the main carbohydrate source (IDF, 1995), followed by incubation at 43 °C for 72 h. Incubation temperature at 43 °C was required for the suppression of the mesophilic *Lactococcus* growth present in the cheese starter culture. To confirm the selectivity of the growth conditions, the morphology of cells from individual colonies was examined by microscope.

### 2.6. Statistical analysis

Statistical analysis was performed using SAS Software version 9.2 (SAS Institute, Cary, NC, USA). Data on milk and cheeses overall composition, cheeses CLA content estimates, and *L. acidophilus* populations were presented as mean  $\pm$  standard deviation (SD).

One-way Analysis of Variance, followed by Tukey post hoc test, was used to determine significant differences ( $P < 0.05$ ) among milk and cheese trials for overall composition. Cheese CLA content estimates and *L. acidophilus* population data were analyzed using Repeated Measures Analysis of Variance to determine significant differences between treatments and ripening time, followed by Tukey test (Bower, 1998a). The equivalent non-parametric tests were applied for results showing non-homogenous variance, according to the Cochran test (Bower, 1997, 1998b). Data on cheeses fatty acids composition was analyzed through Analysis of Variance (ANOVA), followed by Tukey test, considering the following model:  $y_{ijk} = \mu + T_i + P_j + \varepsilon_{ijk}$ , where  $y_{ijk}$  is a single fatty acid,  $\mu$  is the overall mean,  $T_j$  is the treatment,  $P_j$  is the ripening time and  $\varepsilon_{ijk}$  is the residual random error. The interaction among treatment and ripening time ( $T_j * P_j$ ) was tested, and it was excluded from the model because was not significant ( $P > 0.05$ ).

## 3. Results and discussion

### 3.1. Milk and cheese composition and fatty acid profiles

Goats' diets with SBO supplementation increased the fat and total solids content (% w/w, total matter) of milk ( $P < 0.05$ ). Milk from goats fed control and modified diets presented, respectively,  $2.58 \pm 0.17\%$  and  $3.02 \pm 0.07\%$  of fat (mean  $\pm$  SD), and  $2.58 \pm 0.17\%$  and  $3.02 \pm 0.07\%$  of total solids (mean  $\pm$  SD). Milk protein and ash contents (% w/w, total matter) were not affected significantly by the modified diet ( $P < 0.05$ ). These contents were of  $2.73 \pm 0.19$  (control) and of  $2.75 \pm 0.25\%$  (modified) for protein, and of  $0.742 \pm 0.025$  (control) and of  $0.731 \pm 0.018$  (modified) for ash. Similar results concerning changes in milk composition as a consequence of SBO supplementation of goats diet were reported by Bouattour, Casals, Albanell, Such, and Caja (2008), particularly the increase in fat level and the maintenance of protein content.

Mean overall composition (% w/w, total matter) of cheeses T1, T2, T3, and T4 is presented in Table 2. Due to the higher fat content of milk produced by goats fed the modified diet, mean fat content of T3 and T4 cheeses was higher than of T1 and T2 cheeses ( $P < 0.05$ ). Despite their higher total fat content, 10% average, the increased

**Table 2**

Physicochemical and microbiological analysis of Coalho cheeses made with control or modified goats' milk, without or with the addition of *L. acidophilus* (mean  $\pm$  standard deviation).<sup>a</sup>

Analysis <sup>b</sup>	Cheese trials			
	T1	T2	T3	T4
Total solids** (%)	52.25 $\pm$ 1.50 <sup>a</sup>	53.16 $\pm$ 0.97 <sup>a</sup>	53.93 $\pm$ 1.91 <sup>a</sup>	53.25 $\pm$ 1.90 <sup>a</sup>
Fat*** (%)	24.11 $\pm$ 1.45 <sup>a</sup>	23.94 $\pm$ 0.92 <sup>a</sup>	26.39 $\pm$ 0.89 <sup>b</sup>	27.16 $\pm$ 0.61 <sup>b</sup>
Protein** (%)	21.18 $\pm$ 1.17 <sup>a</sup>	21.77 $\pm$ 1.11 <sup>a</sup>	21.26 $\pm$ 1.31 <sup>a</sup>	20.97 $\pm$ 1.02 <sup>a</sup>
Ash** (%)	3.49 $\pm$ 0.27 <sup>a</sup>	3.62 $\pm$ 0.36 <sup>a</sup>	3.54 $\pm$ 0.26 <sup>a</sup>	3.65 $\pm$ 0.16 <sup>a</sup>
Chlorides** (%)	1.42 $\pm$ 0.03 <sup>a</sup>	1.38 $\pm$ 0.25 <sup>a</sup>	1.43 $\pm$ 0.16 <sup>a</sup>	1.53 $\pm$ 0.08 <sup>a</sup>
<i>L. acidophilus</i> *** (log cfu g <sup>-1</sup> )				
Day 1	–	7.12 $\pm$ 0.04 <sup>Aa</sup>	–	7.30 $\pm$ 0.14 <sup>Ab</sup>
Day 15	–	7.59 $\pm$ 0.43 <sup>Aa</sup>	–	7.69 $\pm$ 0.17 <sup>Ba</sup>
Day 30	–	7.39 $\pm$ 0.37 <sup>Aa</sup>	–	7.69 $\pm$ 0.32 <sup>Ba</sup>
Day 45	–	7.55 $\pm$ 0.31 <sup>Aa</sup>	–	7.49 $\pm$ 0.18 <sup>ABa</sup>
Day 60	–	7.31 $\pm$ 0.42 <sup>Aa</sup>	–	7.39 $\pm$ 0.14 <sup>ABa</sup>

<sup>a</sup> Samples were collected on the first day of ripening; cheeses were made from: T1, milk from control diet group; T2, milk from control diet group + *L. acidophilus*; T3, milk from modified diet group; T4, milk from modified diet group + *L. acidophilus*. Different lowercase superscript letters in a same row denote significant differences ( $P < 0.05$ ) between cheese trials, different superscript capital letters in a same column denote significant differences ( $P < 0.01$ ) during storage for a same cheese trial.

<sup>b</sup> For total solids, protein, ash and chloride, duplicate samples from three repeated batches for each trial were analysed; for fat and microbial analyses, triplicate samples from three repeated batches for each trial were analysed.

concentration of CLA, oleic, and linoleic acid, as well as the lower level of total saturated fat in cheeses T3 and T4 (as presented next) improved their quality regarding fatty acid profile, according to the current recommendations on dietary fat (Erkkilä, de Mello, Riséru, & Laaksonen, 2008).

The modified diet with SBO supplementation also resulted in changes in fatty acid profile of cheeses T3 and T4 (Table 3). These cheeses presented reduced concentrations of small and medium chain fatty acids (C6–C10 and C12–C16, respectively), and increased content of long chain fatty acids (>C16). Total saturated fat level (sum of individual saturated FAME) was also lower and, specifically, a markedly lower amount of palmitic acid (C16:0), as well as a higher content of stearic acid (C18:0) and of the unsaturated fatty acids octadecenoic (C18:1), octadecadienoic (C18:2), and octadecatrienoic (C18:3) was observed in the cheeses manufactured with milk from goats receiving SBO. Even though the *trans*-isomers distribution of octadecenoic acid was not detailed through the chromatographic method employed in the present study, the results obtained by Bernard et al. (2009) and Bouattour et al. (2008) showed that the *trans*-11 C18:1 isomer (vaccenic acid) is the major fraction of the increased content of *trans* C18:1 in milk from goats receiving vegetable oils supplementation. Vaccenic acid is an intermediate compound in the biosynthesis of *cis*-9, *trans*-11 CLA by ruminants, and could also be converted into this CLA isomer in the human body, even though its positive effects on human health are not yet well established (Gebauer et al., 2011; Willet & Mozaffarian, 2008). In general, the observed changes in cheese fatty acids profile in the present study are in agreement with those reported by Bouattour et al. (2008) and Matsushita et al. (2007) for milk from goats fed a diet containing the same oil source.

The content of *cis*-9, *trans*-11 CLA in modified milk and in cheeses T3 and T4 was approximately 3-fold higher than the percentages observed in control milk and in cheeses T1 and T2

**Table 3**

Fatty acid composition of cheeses manufactured with milk from control and modified goats' diet, without or with the addition of *L. acidophilus*.

Fatty acid (%, w/w, total FAME) <sup>a</sup>	Cheese trials <sup>b</sup>				SEM <sup>c</sup>	P-value <sup>d</sup>
	T1	T2	T3	T4		
C4:0	1.85 <sup>a</sup>	1.38 <sup>a</sup>	1.61 <sup>a</sup>	1.73 <sup>a</sup>	0.09	ns
C6:0	2.06 <sup>a</sup>	1.88 <sup>a</sup>	1.70 <sup>b</sup>	1.69 <sup>b</sup>	0.05	*
C8:0	2.35 <sup>a</sup>	2.29 <sup>a</sup>	1.77 <sup>b</sup>	1.76 <sup>b</sup>	0.06	**
C10:0	8.94 <sup>a</sup>	8.92 <sup>a</sup>	6.06 <sup>b</sup>	6.03 <sup>b</sup>	0.31	***
C12:0	4.07 <sup>a</sup>	4.06 <sup>a</sup>	2.37 <sup>b</sup>	2.39 <sup>b</sup>	0.18	***
C14:0	11.97 <sup>a</sup>	11.95 <sup>a</sup>	7.44 <sup>b</sup>	7.38 <sup>b</sup>	0.47	***
C14:1	0.13 <sup>a</sup>	0.13 <sup>a</sup>	0.06 <sup>b</sup>	0.07 <sup>b</sup>	0.01	***
C15:0	0.69 <sup>a</sup>	0.71 <sup>a</sup>	0.58 <sup>b</sup>	0.59 <sup>b</sup>	0.01	***
C16:0	30.27 <sup>a</sup>	30.22 <sup>a</sup>	23.88 <sup>b</sup>	23.60 <sup>b</sup>	0.68	***
C16:1	0.43 <sup>a</sup>	0.43 <sup>a</sup>	0.35 <sup>b</sup>	0.35 <sup>b</sup>	0.01	***
C17:0	0.44 <sup>a</sup>	0.44 <sup>a</sup>	0.34 <sup>b</sup>	0.35 <sup>b</sup>	0.01	***
C17:1	0.19 <sup>a</sup>	0.19 <sup>a</sup>	0.10 <sup>b</sup>	0.10 <sup>b</sup>	0.01	***
C18:0	9.11 <sup>a</sup>	9.16 <sup>a</sup>	15.58 <sup>b</sup>	15.38 <sup>b</sup>	0.67	***
C18:1t	1.49 <sup>a</sup>	0.81 <sup>a</sup>	5.49 <sup>b</sup>	5.73 <sup>b</sup>	0.51	***
C18:1n9c	21.38 <sup>a</sup>	21.04 <sup>a</sup>	24.17 <sup>b</sup>	23.92 <sup>b</sup>	0.38	***
C18:2n6t	0.03 <sup>a</sup>	0.04 <sup>a</sup>	0.17 <sup>b</sup>	0.17 <sup>b</sup>	0.02	***
C18:2n6c	1.94 <sup>a</sup>	1.94 <sup>a</sup>	3.12 <sup>b</sup>	3.08 <sup>b</sup>	0.12	***
C18:3	0.28 <sup>a</sup>	0.31 <sup>a</sup>	0.40 <sup>b</sup>	0.43 <sup>b</sup>	0.02	***
C18:2c9t11	0.40 <sup>a</sup>	0.42 <sup>a</sup>	1.40 <sup>b</sup>	1.37 <sup>b</sup>	0.10	***
Other FAME	0.29 <sup>a</sup>	0.28 <sup>a</sup>	0.23 <sup>b</sup>	0.22 <sup>b</sup>	0.01	***
Non-identified	2.98	3.39	3.19	3.62	0.14	ns

<sup>a</sup> Values are the overall mean from one sample from two batches for each trial collected three times throughout the ripening period (days 1, 30 and 60); different lowercase superscript letters in a same row denote significant differences between cheese trials.

<sup>b</sup> T1, milk from control diet group; T2, milk from control diet group + *L. acidophilus*; T3, milk from modified diet group; T4, milk from modified diet group + *L. acidophilus*.

<sup>c</sup> SEM = standard error of means.

<sup>d</sup> P-values are: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; ns, non-significant ( $P > 0.05$ ).

(Table 4). This isomer represents up to 90% of total CLA in milk fat, and the results confirmed some preliminary studies of our research group, in which the inclusion of SBO in the diet increased above 100% (w/w) the CLA levels in goat milk (Bomfim et al., 2006). Minor CLA isomers were not detected by the chromatographic method employed in the present study. Bouattour et al. (2008) and Bernard et al. (2009) also reported a higher CLA content in milk from goats fed diets supplemented with different vegetable oils. In other small ruminants, such as sheep, diet supplementation with a source of linoleic acid also favours markedly increased *cis*-9 *trans*-11 C18:2 contents (Gómez-Cortés et al., 2008; Hervás et al., 2008). In the present study, the average T3 and T4 cheeses estimated CLA content when the whole storage is considered (354.49 mg 100 g<sup>-1</sup> and 350.49 mg 100 g<sup>-1</sup> sample, respectively) was higher than ones obtained by Van Nieuwenhove, Oliszewski, and González (2009) for cheeses made with milk from goats fed on natural pasture during spring and summer seasons in Northeast of Argentina (222.6 mg 100 g<sup>-1</sup>). There was no effect of the manufacturing process on the CLA content of the Coalho cheeses studied, which is in agreement with studies concerning the manufacture of CLA-enriched cheeses using milk from other animal species (Buccioni et al., 2010; Jones et al., 2005; Luna et al., 2008) or milk submitted to more drastic processing conditions (Luna et al., 2005).

The ripening conditions and time employed in our study did not affect significantly ( $P > 0.05$ ) the cheeses CLA content (Table 4), and their overall fatty acids profile (data not shown). Luna, Juárez, and de la Fuente (2007) also reported a negligible effect of ripening on CLA concentration in a study with three Spanish cheeses Protected with Denomination of Origin. On the other hand, Buccioni et al. (2010) verified an increased *cis*-9, *trans*-11 CLA content during the aging of Pecorino Toscano cheese, which was restricted to the first 30 days of ripening.

The CLA content of cheeses in the present study was not influenced by the presence of *L. acidophilus* La5, as shown by the similar

**Table 4**

Effect of goat diet supplementation with vegetable oil on C18:2 *cis*-9, *trans*-11 methyl ester (CLAME) and estimative of C18:2 *cis*-9, *trans*-11 fatty acid (CLA) of goat milk and Coalho cheeses prepared using these milk trials, without or with the addition of *L. acidophilus*.<sup>a</sup>

Milk and cheese <sup>b</sup>	Cheese ripening (days)	CLAME (% w/w, total FAME)	Estimate of CLA (mg 100 g <sup>-1</sup> sample)
Milk			
Control diet	–	0.345 ± 0.038 <sup>a</sup>	8.42 ± 0.902 <sup>a</sup>
Modified diet	–	1.237 ± 0.170 <sup>b</sup>	35.14 ± 4.78 <sup>b</sup>
Cheese trials			
T1	1	0.403 ± 0.031 <sup>A a</sup>	91.60 ± 6.96 <sup>A a</sup>
	30	0.412 ± 0.031 <sup>A a</sup>	96.30 ± 7.08 <sup>A a</sup>
	60	0.401 ± 0.021 <sup>A a</sup>	92.78 ± 4.77 <sup>A a</sup>
T2	1	0.397 ± 0.052 <sup>A a</sup>	89.66 ± 11.55 <sup>A a</sup>
	30	0.436 ± 0.058 <sup>A a</sup>	103.10 ± 13.57 <sup>A a</sup>
	60	0.431 ± 0.053 <sup>A a</sup>	104.49 ± 12.70 <sup>A a</sup>
T3	1	1.39 ± 0.075 <sup>A b</sup>	344.91 ± 18.43 <sup>A b</sup>
	30	1.41 ± 0.018 <sup>A b</sup>	359.72 ± 5.02 <sup>A b</sup>
	60	1.38 ± 0.127 <sup>A b</sup>	358.84 ± 32.63 <sup>A b</sup>
T4	1	1.37 ± 0.113 <sup>A b</sup>	349.67 ± 29.05 <sup>A b</sup>
	30	1.37 ± 0.092 <sup>A b</sup>	356.02 ± 24.01 <sup>A b</sup>
	60	1.38 ± 0.152 <sup>A b</sup>	360.77 ± 39.54 <sup>A b</sup>

<sup>a</sup> Values are the mean ± standard deviation; one sample from two batches for each trial were analysed. Different superscript lowercase letters in a same column denote significant differences ( $P < 0.01$ ) for a same ripening period between trials; different superscript capital letters in a same column denote significant differences ( $P < 0.01$ ) during ripening for a same cheese trial.

<sup>b</sup> Cheeses were made from: T1, milk from control diet group; T2, milk from control diet group + *L. acidophilus*; T3, milk from modified diet group; T4, milk from modified diet group + *L. acidophilus*.

CLA content estimates of non probiotic cheeses T1 and T3 ( $93.56 \pm 5.39$  and  $354.42 \pm 18.47$  mg 100 g<sup>-1</sup>, respectively), and probiotic cheeses T2 and T4 ( $99.08 \pm 12.22$  and  $355.59 \pm 24.90$  mg 100 g<sup>-1</sup>, respectively). The addition of probiotic cultures, including *L. acidophilus*, was reported to enhance CLA levels in dairy products, such as yoghurt (Akalin, Tokoşoğlu, Gönç, & Aycan, 2007), cultured cream (Ekinci, Okur, Ertekin, & Guzel-Seydim, 2008), and Indian fermented milk dahi (Yadav, Jain, & Sinha, 2007). Generally, a fermentation period close to or longer than 4 h is necessary for the processing of these kinds of dairy products, which is probably associated with the improvement in the CLA content by lactic acid bacteria, once the content of C18:2 *cis*-9, *trans*-11 fatty acid tends to be stable throughout the refrigerated storage. For the Coalho cheese-making, bacteria from the starter and probiotic cultures are exposed to optimal growth temperatures during a much shorter period, which would be insufficient to change the content of this fatty acid in the curd.

### 3.2. Probiotic viability in conjugated linoleic acid-enriched Coalho cheeses

Standards requiring a minimum probiotic dose of 6–7 log colony-forming units (cfu) per g or mL of dairy product were established by several food organizations worldwide (Talwalkar & Kailasapathy, 2004). In accordance with Brazilian regulatory standards, probiotic microorganisms should be present in concentrations higher than 8–9 log cfu per daily serving portion of the product ready to consume during the entire shelf life (ANVISA, 2008). Brazilian standards recommend the daily serving portion of 30 g for several types of cheeses (ANVISA, 2003). In the present study, Coalho cheeses T2 and T4 presented *L. acidophilus* populations above 7 log cfu g<sup>-1</sup> during the entire period studied (Table 2), which corresponds to a product containing at least 8.5 log cfu in a serving portion of 30 g. Probiotic populations remained relatively stable during the 60 days period for both cheese trials. Even though cheeses T4 presented a significant increase in the probiotic viability between 1 and 15 days ( $P < 0.01$ ), populations of *L. acidophilus* after 60 days were not significantly different from those observed on day 1 ( $P > 0.01$ ). Also, *L. acidophilus* populations of  $7.15 (\pm 0.12)$  and of  $7.12 (\pm 0.02)$  log cfu g<sup>-1</sup> were recovered on cheese whey, respectively, from control and from modified milk.

The viability of probiotic *Lactobacillus* strains on goat dairy products has been reported as favourable by several authors (Farnsworth, Li, Hendricks, & Guo, 2006; Gomes & Malcata, 1998; Güler-Akin & Akin, 2007; Kongo, Gomes, & Malcata, 2006; Minervini, Bilancia, Siragusa, Gobetti, & Caponio, 2009). One of the pioneer studies with probiotic goat's cheese (Gomes & Malcata, 1998) reported viability of *L. acidophilus* strain Ki around or above 7 log cfu g<sup>-1</sup> in caprine cheeses ripened during 70 days at 6 °C and relative humidity of 92%.

Even though evidence of CLA production by probiotic bacteria, including the *L. acidophilus* La5 strain, has been reported (Akalin et al., 2007; Macouzet, Lee, & Robert, 2009), different types and concentrations of fatty acids in the environment may affect the microbial survival, as observed by Corcoran, Stanton, Fitzgerald, and Ross (2007) for *Lactobacillus rhamnosus* GG in acidic conditions and in the presence of linoleic acid (C18:2, *cis*-9, *cis*-12) and CLA (C18:2, *cis*-9, *trans*-11). In the present study, however, no significant differences were detected between trials T2 and T4, regarding populations of *L. acidophilus* throughout the ripening period ( $P > 0.01$ ), showing that the increased CLA content in Coalho cheese did not affect the viability of this probiotic strain in this kind of product, in both trials during the studied period.

## 4. Conclusion

The present study evaluated the CLA concentration and the viability of a probiotic strain, *L. acidophilus* La5, in caprine Coalho cheese manufactured with naturally CLA-enhanced milk obtained through dietary manipulation. According to the results, the modified milk originated cheeses with a healthier fatty acid profile, presenting an increased CLA, oleic and linoleic acid levels, as well as a lower content of total saturated fat. The higher CLA content (isomer C18:2 *cis*-9, *trans*-11) in cheeses was stable and did not affect the probiotic viability during the 60 days ripening period, which is in accordance with the international standards and the Brazilian legislation for probiotic products. The CLA-enriched probiotic caprine Coalho cheese obtained is proposed as a source of beneficial microorganisms and fatty acids.

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