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Fatty acid profile of milk and Cacioricotta cheese from Italian Simmental cows as affected by dietary flaxseed supplementation

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ABSTRACT

The study aimed to determine the effects of adding flaxseed to the diet on the fatty acid profile of the milk of Italian Simmental cows and on the Cacioricotta cheese thereby produced. The experiment involved 24 Italian Simmental cows divided into 2 groups of 12 animals according to the diet fed: a control diet (CO) with no flaxseed supplementation, and a diet supplemented with whole flaxseed (FS). Milk yield and composition was not significantly changed by diet, whereas saturated fatty acids, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were increased by flaxseed supplementation. Cows fed flaxseed showed higher percentages of long-chain fatty acids: in particular, linolenic acids, mainly represented by C18:3n-3, and n-3 series were higher in the FS group than in the CO group. The percentage of MUFA was higher by about 12% in FS than in CO, mainly due to the contribution of C18:1 *cis*-9. The percentage of conjugated linoleic acid (CLA) in milk was not significantly changed by flaxseed supplementation. Furthermore, atherogenic and thrombogenic indices were lower by about 30 and 16%, respectively, in the FS group compared with the CO group. The fatty acid profile of Cacioricotta cheese produced using Italian Simmental cow milk showed higher levels of MUFA, PUFA, and n-3, and improved atherogenic and thrombogenic indices in FS than in CO, confirming the ability to transfer beneficial molecules from milk into cheese. In particular, cheese-making technology contributed to the increased CLA content in Cacioricotta cheese.
Key words: flaxseed, Italian Simmental cow, fatty acid, Cacioricotta cheese

INTRODUCTION

Milk fat contains substantial concentrations of SFA and relatively low concentrations of MUFA and PUFA; therefore, it has been criticized because it contains a less desirable balance of fatty acids than vegetable fat or fish oil (Kennelly, 1996). Health-conscious consumers have gained awareness that MUFA and PUFA are healthier than SFA; in particular, research has shown several health benefits of n-3 fatty acids (including α-linolenic acid) to humans, including a decrease in the incidence of cancer, cardiovascular diseases, hypertension, and arthritis and an improvement in visual ability (Simopoulos, 1996; Wright et al., 1998). Therefore, consumer demand today is oriented toward dairy products with a valuable fatty acids profile to meet their health concerns.

In recent years, several experiments conducted on dairy cows have shown that a supplementation of oilseeds (rich in n-3 fatty acids) such as flaxseed, rapeseed, or soybean is an effective strategy for improving the nutritional value of milk fat (Shingfield et al., 2008) through an increase of the levels of PUFA and MUFA. Few studies have investigated the fatty acid profile of cheese made from milk produced from livestock receiving oilseed supplementation; the majority have involved cheese from small ruminant milk (Luna et al., 2005; Nudda et al., 2005; Gòmez-Cortés et al., 2009, Mele et al., 2011) whereas less research has been conducted on cheese produced from Friesian cows fed oilseeds (Dhiman et al., 1999; Caroprese et al., 2013; Cattani et al., 2014).

Several factors play a role in the efficiency with which milk components are transferred into cheese, and these are related to the feeding regimen of the animals (Banks et al., 1986), milk features, and cheese-making conditions (Lucey and Kelly, 1994). In particular, milk-processing temperature may affect lipid composition of cheese; high processing temperatures and addition of whey protein concentrates have been found to increase the formation of CLA in the processed cheese as the result of oxidation processes (Shantha et al., 1992; Garcia-Lopez et al., 1994). In southern Italy, a typical and traditional cheese is Cacioricotta cheese, which is usually produced from goat milk; this type of cheese is made using an unusual technology—a high heat treatment at 90°C of whole milk that allows the recovery

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of whey proteins in the curd, giving high cheese yields (Albenzio et al., 2006).

To the best of our knowledge, no studies have been reported on the production of Cacioricotta cheese from bovine milk. It is unclear whether the unusual cheese-making technology could affect the transfer of valuable nutritional fat components of milk into Cacioricotta cheese. In the light of this, the present paper aimed to evaluate the effects of flaxseed supplementation on the composition and the fatty acid profile of milk and Cacioricotta cheese from Italian Simmental cows.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted in June and July 2014 in a dairy farm located in Cisternino (Brindisi, Apulian region, Italy). The experiment involved 24 Italian Simmental cows during mid lactation (175 ± 12 DIM); animals were homogeneous for age (46 ± 6 mo), BW (475 ± 18 kg), BCS (3.68 ± 0.5), parity (2.58 ± 0.28), milk production (19.8 ± 0.80 kg/d), milk fat content (3.72 ± 0.5%), milk protein content (3.35 ± 0.1%), and fatty acid composition. Animals were assigned randomly to 1 of 2 groups subjected to different diets: (1) the control group (**CO**) received a diet based on 9.5 kg of concentrate mainly constituted by corn (51%), soy (22%), barley flour (4%), and bran (4.8%), 5 kg of corn grains, and 6.5 kg of vetch and oat hay; (2) the flaxseed group (**FS**) received the same diet but 1 kg of concentrate was substituted with the same amount of whole flaxseed (Lin Tech, Tecnozoo srl, Torreselle di Piombino Dese, Italy). The chemical composition of the diets is reported in Table 1.

Table 1. Ingredient and chemical composition of the experimental diets (% on DM basis)

	Diet[1]	
Item	CO	FS
Concentrate	45.00	40.24
Corn	24.00	24.00
Vetch and oat hay	31.00	31.00
Whole flaxseed	—	4.76
Ether extract	2.67	4.72
CP	15.38	15.41
ADF	25.43	25.45
NDF	42.60	42.48
ADL	3.34	3.45
NE_L[2] (Mcal/kg)	1.56	1.68

[1]CO = control group; FS = flaxseed group.

[2]Calculated according to NRC (2001).

The experiment lasted 7 wk; the first 2 wk were considered an adaptation period and measurements were made during the last 5 wk. Cows were housed in straw-bedded barns with free access to water and were fed twice daily (at 0800 and 1600 h). The total amount of flaxseed was given before the morning feeding to each cow of the FS group and we verified that each animal consumed the total quantity of supplement given. Cows were milked mechanically twice daily at 0600 and 1800 h, and milk production was recorded at each milking. Milk collection was done once a week on the same day throughout the experiment. Individual milk samples were obtained by mixing milk from the morning and afternoon milkings in an amount proportional to milk yield. Individual milk samples were stored under refrigeration and transferred to the laboratory for analyses.

At the end of the wk 6 and 7 of the experiment, bulk milk was collected and pooled from the evening and morning milkings from each experimental group. Pooled milk from each group was divided into 2 aliquots and processed to Cacioricotta cheese using the following protocol: raw milk was heated at 90°C, held for 2 min, and then cooled to 40°C. Then, 1% of saturated brine (23% NaCl, 0.06% $CaCl_2$) was added. Subsequently, 150 g/100 L of rennet (Chr. Hansen s.p.a., Parma, Italy) containing 77% chymosin was added, and curdling was obtained in about 10 min. The subsequent steps were cutting of the coagulum to rice-grain size, putting the curd into reed containers, and manual pressing of the curd to facilitate the draining off of the whey. The curd was held at controlled temperature (22°C) for 24 h, dry-salted for 1 d, and then ripened for 7 d at 12°C and 80% relative humidity. At the end of the ripening time, cheeses were transferred to the laboratory under refrigeration, and 3 cheeses from each cheese-making were analyzed in triplicate.

Analyses of Milk

Individual and bulk milk samples were analyzed for fat, protein, casein, and lactose contents (MilkoScan FT 120; Foss Electric A/S, Hillerød, Denmark), and SCC (Fossomatic Minor, Foss-Electric A/S). Individual and bulk milk renneting characteristics (clotting time, rate of clot formation, and clot firmness after 30 min) were measured using a Formagraph (Foss Electric A/S).

Fatty acids extraction from milk samples was performed as described by Feng et al. (2004), with some modifications. Briefly, 30 mL of bulk milk was centrifuged at 17,800 × *g* for 45 min at 4°C. Then, 1.0 g of the fat layer was transferred into a microtube, left at room temperature for 30 min, and centrifuged at 19,300 × *g* for 40 min at 20°C. Fatty acids methyl esters were

obtained as described in IDF (2002); 100 mg of the upper layer was placed into a 16- × 25-mm screw-capped Pyrex tube to which 5 mL of hexane and 0.2 mL of 2 *N* methanolic KOH were added. The tube was vortexed and left to stand for 5 min in the dark; then, 0.5 g of $NaHSO_4 \cdot H_2O$ was added. The hexane layer, containing the FAME, was placed into a GC vial. The vial was capped and placed at −20°C until GC analysis. The fatty acid composition of milk extracts was determined by capillary GC on a HP-88, 100 m × 0.25 mm × 0.20 μm capillary column (Agilent Technologies Inc., Santa Clara, CA) installed on an Agilent Technologies 6890N gas chromatograph equipped with a flame-ionization detector and a split injection. The initial oven temperature was 70°C, held for 4 min, subsequently increased to 175°C at a rate of 13°C min^{-1}, and held for 27 min, increased to 215°C at a rate of 4°C min^{-1}, and then held for 45 min. Helium was used as the carrier gas and the column head pressure was 175 kPa. Both the injector and the detector were set at 250°C. The split ratio was 20:1. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards (FIM-FAME-7-Mix, Matreya LLC, Pleasant Gap, PA), and C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:2 *cis*-9,*cis*-11, C18:2 *trans*-9,*trans*-11, and C18:2 *trans*-10,*cis*-12 (Matreya LLC) and peak areas were quantified using Agilent Chemstation software.

Atherogenic and thrombogenic indices were calculated according to formulas from Ulbricht and Southgate (1991): atherogenic index = (C12:0 + 4 × C14:0 + C16:0)/[Σ MUFA + Σ PUFA(n-6) and (n-3)]; thrombogenic index = (C14:0 + C16:0 + C18:0)/[0.5 × Σ MUFA + 0.5 × Σ PUFA(n-6) + 3 × Σ PUFA(n-3) + (n-3)/(n-6)].

Analyses of Cacioricotta Cheese

The DM content of cheese was determined according to International Dairy Federation method (IDF, 1986). Total nitrogen was determined as described by Gripon et al. (1975), and fat was determined by the Soxhlet method using diethyl ether.

Extraction of fatty acids from cheese samples was performed as described by O'Fallon et al. (2007). Briefly, 1.0 g of sample was placed into a 16- × 125-mm screw-capped Pyrex tube to which C13:0 internal standard (0.5 mg of C13:0/mL of methanol), 0.7 mL of 10 *N* KOH, and 5.3 mL of methanol were added. The tube was incubated in a 55°C water bath for 1.5 h with shaking by hand every 20 min. After cooling the tube below room temperature, 0.58 mL of 24 *N* H_2SO_4 was added. The tube was mixed by inversion and incubated again in a 55°C water bath for 1.5 h with shaking by hand every 20 min. Then, the tube was cooled in a cold tap water bath, 3 mL of hexane was added, and the tube was vortexed for 5 min. The tube was then centrifuged at 500 × *g* for 5 min, and the hexane layer, containing the FAME, was placed into a GC vial. The vial was capped and placed at −20°C until GC analysis according to the previous method described for milk extracts.

Statistical Analysis

All variables were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Data were processed using ANOVA for repeated measures (SAS Institute, 2011), with flaxseed supplementation, time of sampling, and their interactions as repeated factors. Where significant effects were found ($P < 0.05$), Student's *t*-test was used to locate significant differences between means. The interaction of treatment and time was not significant for milk yield, milk and cheese composition, or for fatty acid content of milk and cheese; therefore, mean values for the mentioned parameters are presented.

RESULTS AND DISCUSSION

The chemical composition of milk obtained from Italian Simmental cows subjected to different diets was not significantly influenced by flaxseed supplementation. Mean percentages of fat, protein, lactose, and casein were 3.9 ± 0.5, 3.2 ± 0.1, 4.8 ± 0.1, and 2.5 ± 0.1% respectively. Milk yield was not affected by diet although it exhibited a lower value in the FS group (24.06 ± 1.2 kg/d) than in the CO group (26.68 ± 1.2 kg/d). Gonthier at al. (2005) also found that cows fed flaxseed diets produced 1.8 kg less milk than those fed the control diet although differences were not significant; moreover, diet supplementation with whole flaxseed generally has little effect on milk production of cows in the mid stage of lactation (Petit, 2010). The effect of linseed supplementation on milk components is still controversial although several studies have reported that flaxseed supplementation did not change either the fat (Gonthier et al., 2005) or the protein concentration of milk (Petit et al., 2004; Martin et al., 2008). Somatic cell count was not influenced by the tested diets, and the mean values detected in CO and FS groups were 126.4 ± 23.19 and 127.22 ± 22.13 × 10^3 cells/mL of milk, respectively. Milk SCC has generally not been affected by flaxseed supplementation (Martin et al., 2008) although few data have been published on this topic. Some evidence suggests that feeding plant oils rich in n-3 fatty acids alters the production

of cytokines and functional properties of macrophages, lymphocytes, and other immunocompetent cells as shown by a reduced proliferative response of activated peripheral blood mononuclear cells of dairy cows fed whole flaxseed (Lessard et al., 2003).

Milk coagulating ability was studied by measuring coagulation time, rate of clot formation, and clot firmness, which were not influenced by the tested effect: coagulation time was 11.34 ± 0.58 (CO) and 10.8 ± 0.47 min (FS); rate of clot formation was 3.97 ± 0.38 (CO) and 3.62 ± 0.32 min (FS); and curd firmness after 30 min was 28.14 ± 1.65 (CO) and 29.37 ± 1.42 mm (FS).

Mean values of fatty acids in milk from cows subjected to flaxseed supplementation are presented in Table 2. Short-, medium-, and long-chain fatty acids were influenced by diet, confirming that supplemental fats in the diet are able to influence fatty acid percentages in milk fat (Caroprese et al., 2010). In particular, all fatty acids ranging from C4:0 to C12:0 were lower in FS than in CO, whereas of the medium-chain fatty acids, only C14:0 and C16:0 were lower in the FS group. It has been reported that long-chain fatty acids are powerful inhibitors of acetyl-CoA carboxylase (Chilliard and Ferlay, 2004) involved in the de novo synthesis of milk fat; supplemental PUFA have been shown to reduce the concentrations of short- and medium-chain fatty acids in milk (Zhang et al., 2006). Focusing on the long-chain fatty acid group, the linolenic acids, mainly represented by C18:3n-3, were about 33% higher in FS than in CO and were derived from the increased supply of dietary long-chain fatty acids. It is reported that C18:3n-3 acid (representing about 55% of fatty acids in flaxseed) induces a large number of intermediaries, but CLA *cis*-9,*trans*-11 production does not seem to occur (Petit, 2010). Conjugated linoleic acid synthesis is attributed to biohydrogenation by ruminal bacteria, especially *Butyrivibrio fibrisolvens*, and later to the uptake of intermediates from the udder, which are desaturated by Δ^9-desaturase. Contents of CLA were numerically, if not significantly, higher in milk from cows fed flaxseed supplementation, according to Cattani et al. (2014). Also, C18:1 *trans*-11 and C18:2 were higher in the FS group, confirming the hypothesis that a complex system of intermediates and products of rumen biohydrogenation of PUFA is delivered by the fat supplement of the diet. Furthermore, higher levels of C18:0 and C18:1 *cis*-9 were found in milk from the FS group compared with the CO group. It has been reported that diets rich in vegetable oils or seeds lead to an increase in stearic acid produced in the rumen, which is then transformed, in part, into oleic acid in the udder (Chilliard and Ferlay, 2004).

Nutritional indices of milk from Italian Simmental cows subjected to different diets are presented in Table 3. Saturated fatty acids, MUFA, and PUFA were affected by diet, with SFA being lower and MUFA and PUFA being higher in FS than in CO; consequently, the ratio of PUFA to SFA was significantly higher in the same group. The MUFA and n-3 fatty acids were higher by about 12 and 24%, respectively, in FS than in CO, mainly due to the contributions of C18:1 *cis*-9 and C18:3n-3. It is well known that the UFA, in particular C18:1 *cis*-9, and PUFA, including CLA, have a protective effect against cardiovascular diseases (Williams, 2000). The ratio of n-6 to n-3 was lower in the FS group, and the atherogenic and thrombogenic indices were lower by about 30 and 16%, respectively, in the same group compared with the control milk. In a previous study, Caroprese et al. (2010) found that linseed supplementation in cow was able to lower the atherogenic index by 13% and the thrombogenic index by 8%.

The chemical parameters of Cacioricotta cheeses from CO and FS groups did not differ significantly, and the mean values for moisture, protein, and fat contents were 67.29 ± 0.16%, 29.9 ± 0.2%, and 24.68 ± 0.58%, respectively, after 7 d of ripening.

The fatty acid composition of Cacioricotta cheeses from milk of Italian Simmental cows subjected to different diets is reported in Table 4. In this study, C18:0,

Table 2. Mean fatty acid (FA) composition (%) of milk from Italian Simmental cows subjected to different diets

	Diet[1]			Effect, *P*-value
FA/group	CO	FS	SEM	Treatment
Short-chain FA[2]	16.130	14.380	0.170	*
C4:0	5.181	4.891	0.121	**
C6:0	2.810	2.582	0.062	*
C8:0	1.534	1.353	0.043	*
C10:0	3.232	2.750	0.131	*
C12:0	3.481	2.942	0.142	*
Medium-chain FA[3]	43.940	40.680	0.300	*
C14:0	11.93	10.4	0.36	*
C16:0	28.33	26.62	0.54	*
Long-chain FA[4]	39.439	44.828	0.100	*
C18:0	11.560	12.860	0.340	***
C18:1 *cis*-9	22.600	25.020	0.680	**
C18:1 *trans*	0.710	1.160	0.070	**
C18:2	2.760	3.160	0.060	**
C18:3	0.66	0.99	0.003	**
CLA	0.153	0.169	0.04	NS

[1]CO = control group; FS = flaxseed group.

[2]Sum of C4:0 to C13:0.

[3]Sum of C14:0 to C17:1.

[4]Longer than C17; C18:1 *trans* = sum of C18:1 *trans*-9 and C18:1 *trans*-11; C18:2 = sum of C18:2 *trans*-9,*trans*-12 and C18:2 *cis*-9,*cis*-12; C18:3 = sum of C18:3n-3 and C18:3n-6; CLA = sum of CLA *cis*-9,*trans*-11, CLA *trans*-10,*cis*-12, and CLA *trans*-9,*trans*-11.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3. Saturated fatty acids, MUFA, PUFA, n-6, and n-3 (%), and principal nutritional indices of milk from Italian Simmental cows subjected to different diets

	Diet[2]			Effect, *P*-value
Item[1]	CO	FS	SEM	Treatment
SFA	64.660	60.330	1.170	**
MUFA	23.160	26.160	1.100	**
PUFA	4.431	5.872	0.171	*
PUFA:SFA	0.070	0.080	0.003	**
n-6	3.751	4.320	0.141	*
n-3	0.712	0.933	0.040	***
n-6:n-3	5.240	4.712	0.190	***
Atherogenic index	2.910	2.042	0.170	**
Thrombogenic index	3.310	2.770	0.130	**

[1]Atherogenic index (C12:0 + 4 × C14:0 + C16:0)/[Σ MUFA + Σ PUFA(n-6) and (n-3)]; thrombogenic index = (C14:0 + C16:0 + C18:0)/[0.5 × Σ MUFA + 0.5 × Σ PUFA(n-6) + 3 × Σ PUFA(n-3) + (n-3)/(n-6)].

[2]CO = control group; FS = flaxseed group.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

C18:1 *cis*-9, C18:2, C18:3n-3, and C22:1, were higher, whereas C14:0 and C16:0 were lower in FS cheese, demonstrating the potential to improve the cheese fatty acid profile by modulating the fat supplement of the diet in lactating cow. It is reported that the fat content of cheese is primarily influenced by the fat content of the original milk, whereas the cheese-making technology is considered to be of minor importance (Lucas et al., 2006).

Higher levels of CLA *cis*-9,*trans*-11 and CLA *trans*-9,*trans*-11 were found in the FS cheese compared with the CO cheese, which was ascribed both to higher levels of precursors of CLA in the milk and to the processing technology of Cacioricotta cheese. Ha et al. (1989) proposed a mechanism for the formation of CLA in dairy products that involves free radical oxidation of linoleic or linolenic acid during cheese processing. In particular, the application of heat enhances the formation of linoleic acid radicals and increases CLA content during the production of natural and process cheeses (Shantha et al., 1992; Garcia-Lopez et al., 1994). The enhancement of CLA formation at elevated temperatures has been observed in preparing process cheese (Shantha et al., 1992; Garcia-Lopez et al., 1994) and ghee, an Indian clarified butter product (Aneja and Murthi, 1990). Cacioricotta cheese-making requires heating the milk to about 90°C to denature the whey proteins with the aim of entrapping them in the coagulum; therefore, the heating step could have played a role in the increase of CLA content in Cacioricotta cheese. Ha et al. (1989) reported that lactalbumin- and lactoglobulin-enriched cheeses contain significantly greater amounts of CLA compared with cheeses not enriched. Under the anaerobic conditions occurring during cheese aging, oxidation of linoleic acid in glycerides or phospholipids may be initiated to form an allyl radical; the radical would be stabilized through the formation of its resonance form, which requires hydrogens to form a conjugated double-bond system.

The mean values of nutritional indices of Cacioricotta cheeses from milk of Italian Simmental cows subjected to different diets are reported in Table 5. Saturated fatty acid contents were lower, whereas contents of MUFA, PUFA, and n-3 were higher in cheese from the FS group compared with the CO group. Atherogenic and thrombogenic indices and the ratio of n-6 to n-3 were lower in FS, confirming the improvement of nutritional features of cheese obtained from milk of cows supplemented with flaxseed. In particular, the balance in the diet between n-6 and n-3 PUFA is involved in the prevention of many diseases such as coronary artery disease (Williams, 2000). Moreover, C18:3n-3 and CLA represented valuable nutritional components in Cacioricotta cheese.

Table 4. Mean fatty acid composition (%) of cheese from milk of Italian Simmental cows subjected to different diets

	Diet[2]			Effect, *P*-value
Fatty acid[1]	CO	FS	SEM	Treatment
C4:0	1.700	1.340	0.160	NS
C6:0	1.180	1.041	0.120	NS
C8:0	0.820	0.751	0.060	NS
C10:0	2.100	1.940	0.100	NS
C12:0	2.784	2.590	0.070	NS
C14:0	11.140	10.570	0.140	*
C14:1	0.851	0.810	0.030	NS
C15:0	1.281	1.240	0.080	NS
C16:0	30.460	29.170	0.110	**
C16:1	1.370	1.330	0.012	NS
C17:0	0.681	0.652	0.006	NS
C18:0	12.120	13.021	0.260	*
C18:1 *trans*-9	0.071	0.082	0.003	NS
C18:1 *trans*-11	2.652	2.143	0.250	NS
C18:1 *cis*-9	25.210	27.430	0.090	***
C18:2	2.800	3.142	0.020	*
C20:0	0.200	0.210	0.009	NS
C18:3n-6	0.050	0.057	0.001	NS
C18:3n-3	0.490	0.751	0.004	***
C20:1	0.140	0.070	0.003	NS
CLA	0.574	0.916	0.013	***
C20:2	0.012	0.011	0.001	NS
C22:1	0.110	0.230	0.04	*
C20:4	0.123	0.111	0.002	NS
C20:5	0.080	0.100	0.003	NS

[1]C18:2 = sum of C18:2 *trans*-9,*trans*-12 and C18:2 *cis*-9,*cis*-12; C18:3 = sum of C18:3n-3 and C18:3n-6; CLA = sum of CLA *cis*-9,*trans*-11, CLA *trans*-10,*cis*-12, CLA *trans*-9,*trans*-11.

[2]CO = control group; FS = flaxseed group.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 5. Saturated fatty acids, MUFA, PUFA, n-6, and n-3 (%), and principal nutritional indices of cheese from milk of Italian Simmental cows subjected to different diets

	Diet[2]			Effect, *P*-value
Item[1]	CO	FS	SEM	Treatment
SFA	62.681	61.223	0.180	**
MUFA	27.931	29.672	0.280	*
PUFA	4.481	4.805	0.09	*
PUFA:SFA	0.070	0.070	0.002	NS
n-6	3.900	3.960	0.09	NS
n-3	0.612	0.902	0.010	***
n-6:n-3	6.371	4.412	0.150	***
Atherogenic index	2.400	2.140	0.030	**
Thrombogenic index	3.010	2.630	0.020	***

[1]Atherogenic index (C12:0 + 4 × C14:0 + C16:0)/[Σ MUFA + Σ PUFA(n-6) and (n-3)]; thrombogenic index = (C14:0 + C16:0 + C18:0)/[0.5 × Σ MUFA + 0.5 × Σ PUFA(n-6) + 3 × Σ PUFA(n-3) + (n-3)/(n-6)].

[2]CO = control group; FS = flaxseed group.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

CONCLUSIONS

Supplementation of flaxseed to Italian Simmental dairy cows did not affect milk or Cacioricotta cheese characteristics in terms of principal composition. However, PUFA from the flaxseed supplement influenced the fatty acid composition of milk, resulting in higher levels of C18:0, C18:1 *cis*-9, C18:2, and C18:3n-3. The improvement of the fatty acid profile of Cacioricotta cheese obtained from FS milk was ascribed to the improved fatty acid profile of the original milk. In particular, the increased levels of CLA in FS cheese were attributed to the heat treatment of the milk that led to the retention of whey proteins in the cheese curd.

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