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Extruded Linseed Supplementation in Dairy Goat Diet: Effects on Productive Performance and Fatty Acid Profile of Bulk Milk, Fresh and Ripened Cheese

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Abstract: Aim of the study was to evaluate the effects of diet supplementation with Extruded Linseed (EL) on goat milk yield and gross composition and Fatty Acid (FA) composition of milk, fresh and ripened cheese. During a 10 weeks period, 26 mid-lactating Saanen goats were divided into two balanced groups and fed a total mixed ration supplemented (EL group) or not (C group, control) with 3 g/100 g diet of rumen unprotected EL. Bulk milk samples, fresh and ripened cheese were analysed for fat, protein, lactose and FA profile. Results showed that milk fat was 3.1 g kg^{-1} higher in the EL group. Milk, fresh and ripened cheeses showed similar responses in FA composition. Dietary inclusion of EL enhanced the concentrations of caprylic, capric and stearic acids, total trans-octadecenoic acids, the majority of non-conjugated octadecadienoic acids and α-linolenic acid. Significant reductions were observed for palmitic acid, iso branched-chain FA, cis-9 monounsaturated FA, the sum of c9t11+t7c9+t8c10 conjugated linoleic acid isomers, linoleic acid and other long-chain n6 FA. The n6/n3 FA ratio and the estimated Δ9-desaturase activity were also significantly reduced. No significant influence of EL supplementation was instead observed for total polyunsaturated, aiso branched-chain and very long-chain n3 FA. The positive effects of the EL inclusion on the nutritional quality of milk and cheese lipids (lower hypercholesterolemic saturated FA and n6/n3 FA ratio and higher caprylic, capric and α-linolenic acids contents) were however accompanied by reduced concentrations of potentially beneficial FA such as 14-methylpentadecanoic (C16 iso), oleic and conjugated linoleic acids.

Key words: Goat, extruded linseed, fatty acids, dairy products, ratio

INTRODUCTION

Vegetable lipids have the potential to considerably influence the nutritional quality of the lipid fraction of milk and cheese. Many interacting factors can determine variable effects of the lipid supplementation on animal performance, extent of ruminal Biohydrogenation (BH) and nutritional, sensorial and technological properties of dairy products (Chilliard and Ferlay, 2004), the source and presentation form of fat (e.g., free oil vs. seeds from rapeseed, sunflower, cottonseed, soybean, linseed, etc.), the processing (e.g., whole/raw material vs. physically, chemically or technologically treated material), the amount included in the diet, the degree of rumen protection and the composition of the basal diet (e.g., forage:concentrate ratio F:C, forage type, starch content).

Regarding milk Fatty Acid (FA) profile, some vegetable lipids have been shown to be effective both in lowering the concentration of Hypercholesterolemic Saturated Fatty Acids (HSFA; lauric-C12:0, myristic-C14:0 and palmitic-C16:0 acids) and increasing the concentration of specific ruminal BH intermediates (e.g., vaccenic-VA, C18:1 t11 and rumenic-RA, C18:2 c9t11 acids) characterized by putative beneficial effects for human health (Chilliard *et al*., 2007; Steijns, 2008).

Linseed provides high amounts of dietary α-linolenic acid (C18:3 c9c12c15, ALA). This usually allows a raise in milk concentration of total n3 FA (essentially ascribed to increases in ALA) and a decrease in the n6/n3 FA ratio which consequently determine additional favourable health effects such as normal embryogenesis and brain development and protection against cancer, cardiovascular and neurodegenerative diseases (Barcelo-Coblijn and Murphy, 2009; Kouba and Mourot, 2011; Simopoulos, 2011).

In the last few years, an increasing number of studies attempted to evaluate the use of linseed supplemented diets in lactating goats. Different forms of linseed have been tested: oils (Bernard *et al*., 2009a, b; Li *et al*., 2012; Marin *et al*., 2011), cakes (Nudda *et al*., 2006) whole seeds (Dehareng *et al*., 2009) even forms protected from ruminal metabolism (Bernard *et al*., 2005). Despite the fact that

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unprotected Extruded Linseed (EL) is the most widely form used in diets for ruminants, information is still scant on its effects on productive efficiency and milk quality in dairy goats. The few available studies tested extruded mixtures of seeds (linseed combined with wheat or sunflower seeds) or pure EL only in short time periods of lipid supplementation (Battacone *et al*., 2007; Chilliard *et al*., 2004, 2005; Dehareng *et al*., 2009).

The aim of the present study was to give further information on the effects of unprotected EL fed to mid-lactating dairy goats in a long term trial on milk yield, gross composition and FA profile of milk fat. As in Mediterranean countries, goat milk is mainly processed into cheese, the effects of EL supplementation on FA profile of fresh and 30 days ripened cheeses were also assessed.

MATERIALS AND METHODS

Animals and dietary treatments: The experiment was carried out in a farm located in North Italy (latitude: 45°30'22"N; longitude: 09°31'48"E; altitude: 110 masl) from 26 June to 31 August, 2012. Twenty six terziparous Saanen goats in mid-lactation were selected from 80 lactating goats and allocated to two balanced groups of 13 animals each, according to their stage of lactation (119±2 days in milk), milk yield (2.3±0.63 kg/head/day), milk gross composition (fat, protein and lactose contents), FA profile of milk fat and body condition score (2.7±0.30) (Hervieu and Morand-Fehr, 1999). The selected goats were randomly assigned to two different experimental diets which were formulated to meet their nutrient requirements (NRC, 2007). The goats were then moved to individual pens with free access to water and mineralized salt blocks.

During the 10 weeks experimental period, one group of goats (C group, control) was fed a Total Mixed Ration (TMR) composed of rye silage, concentrate (containing: wheat middlings, soybean meal, corn, partially dehulled sunflower meal, soybean hulls, sorghum, calcium carbonate, cane molasses, sodium hydrogen carbonate, dicalcium/monocalcium phosphate, sodium chloride), maize meal, soybean meal and mixed hay in the following quantities: 1.8, 0.6, 0.5, 0.4 and 0.4 kg/head/day, respectively.

The other group (EL group) was offered an identical TMR with the addition of 0.08 kg/head/day ruminal unprotected EL. Feeds were provided in equal amounts immediately after the morning (05:30 h) and afternoon (17:30 h) milkings. Feed refusals were controlled once a week for Dry Matter Intake (DMI) estimation.

Feed sampling and analysis: Representative samples of the TMR and EL were analysed for Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), ash, Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) (AOAC, 2000). Lipid extraction and FA transesterification were performed as reported by Alves *et al*. (2008). Fatty Acid Methyl Esters (FAME) were determined as described by Renna *et al*. (2012).

Milk sampling and analysis: Bulk milk yield was recorded and bulk milk samples were collected, once a week throughout the trial after a 3 weeks period of adaptation to the diets (5-25 June, 2012).

Daily milk yields were recorded using graduated measuring cylinders attached to milking units. Bulk milk samples from the morning milkings were divided into two subsamples: one (50 mL) was stored at 4°C and immediately analysed for fat, protein and lactose (MilkoScan FT 6000, Foss Electric, Hillerod, Denmark) while the other (250 mL) was frozen at -80°C and successively analysed for FA composition according to Renna *et al*. (2012). Fatty acids were quantified by the amount of recovered internal standard (nonanoic acid) and expressed in relative concentrations as g/100 g of total identified FA.

Cheese-making procedure: Separate bulk milk tanks (one per treatment) were used for cheese-making. Milk from the morning milking was collected in plastic tubes and added with starters (1:1:1:1 mixture of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*). After 3 h, the milk was coagulated at 26°C with the addition of 1 mL of goat liquid rennet and 2 mg of *Penicillium candidum* pure culture for rind flowering during ripening. After 24 h rest in whey at 26°C and 80% Relative Humidity (RH), the curd was transferred into plastic food crates covered with linen cloths. After 4 h the curd was salted and moulded in cylindrical forms (7 cm diameter and 8 cm height). Both fresh and ripened cheeses (about 120 and 230 g weight, respectively) were produced. Ripened cheeses matured for 30 days in storage bays at 13°C and 70% RH and were turned every other day.

Cheese sampling and analysis: Cheese-making occurred ten times during the trial with the same time schedule used for milk yield recording and milk sampling. Fresh and ripened cheese samples were collected 1 h after cheese-making and after ripening, respectively. The samples were immediately frozen at -80°C until analysed for their FA composition. The rind of the ripened cheeses was removed before analysis as it is not habitually

consumed. Cheese total lipids were extracted according to IDF (1986). Fatty acid methyl esters were prepared according to Chistopherson and Glass (1969) and determined as for milk analysis.

Statistical analysis: The statistical analysis was performed using IBM SPSS Statistics V. 20 for Windows (SPSS Inc., Chicago, IL, USA). Milk yield and gross composition data were submitted to an independent samples Student's t-test. Milk and cheese FA data were submitted to a two-way Analysis of Variance (ANOVA); the model included the effects of dietary treatment, dairy product and their interaction. The Kolmogorov-Smirnov test was used to check dependent variables for normality. The assumption of equal variances was assessed by the Levene's homogeneity of variance test. Pairwise comparisons were performed to test the difference between dairy products' pair of means by using the Tukey's test. Correlations were performed by computing Pearson's correlation coefficients. Significance was declared at $p \leq 0.05$.

RESULTS

Diets composition: Main nutrients and FA compositions of the diets are presented in Table 1. The amount of main nutrients and the Net Energy for lactation (NE_L) were comparable between diets as well as the relative percentage of FA groups on total detected FA (saturated fatty acids-SFA: 18.85 and 16.62 g/100 g; monounsaturated fatty acids-MUFA: 21.40 and 20.81 g/100 g; polyunsaturated fatty acids-PUFA: 59.75 and 62.57 g/100 g in C and EL diets, respectively). Feed ingredients were mainly rich in linoleic acid (C18:2 c9c12, LA) which consequently resulted the most abundant FA in both diets (about 50 and 42 g/100 g of total FA for C and EL diets, respectively). The linseed supplementation noticeably enriched the ALA content of the EL diet, determining a shift in the individual FA composition of the experimental TMR. As a result, the second most abundant FA in the EL diet was ALA which showed a comparable content (about 20 g/100 g of total FA) to that of oleic acid (C18:1 c9, OA). Conversely, the C diet was noticeably richer in OA than ALA: 20 and 9.5 g/100 g of total FA, respectively).

Dry matter and fatty acids intakes: The EL supplementation did not influence the DMI of goats (2.30 and 2.37 kg DM/head/day for the C and EL groups, respectively). The estimated daily intakes of dietary FA groups and individual FA are shown in Table 2. The inclusion of EL increased the daily ingestion of total FA

Table 1: Ingredients (% DM), main nutrients (g/kg DM, unless otherwise stated) and fatty acid composition (mg/100 g DM) of control and extruded linseed experimental diets

Diets	C diet	EL diet
Ingredients		
Rye silage	28.10	27.30
Maize meal	19.00	18.60
Concentrate	22.80	22.10
Soybean meal	15.20	14.70
Mixed hay	14.80	14.30
Extruded linseed[a, b]	-	3.00
Main nutrients		
DM (g kg^{-1})	622.00	641.00
Ash	78.00	79.00
CP	148.00	150.00
EE	30.00	40.00
NDF	385.00	386.00
ADF	215.00	214.00
NSC[c]	359.00	345.00
NE_L (MJ kg^{-1} DM)	6.30	6.30
Fatty acid composition		
C12:0	0.85	0.85
C14:0	6.85	7.29
C16:0	563.67	619.82
C16:1 c9	6.46	7.02
C18:0	83.64	119.57
C18:1 c9 (OA)	716.72	912.09
C18:1 c11	40.97	40.97
C18:2 c9c12 (LA)	1795.69	1959.83
C18:3 c9c12c15 (ALA)	337.79	927.64
C20:0	18.12	19.52
ΣSFA	673.12	767.05
ΣMUFA	764.14	960.08
ΣPUFA	2133.48	2887.47
TFA	3570.74	4614.60

DM: Dry Matter; C: Control diet; EL: Extruded Linseed diet; CP: Crude Protein; EE: Ether Extract; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; NSC: Non-Structural Carbohydrates; NE_L: Net Energy for lactation; OA: Oleic Acid; LA: Linoleic Acid; ALA: α-Linolenic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids. [a]Main nutrients (g/kg DM, unless otherwise stated): DM, 900 g kg^{-1}; Ash, 57; CP, 225; EE, 348; NDF, 267; ADF, 132; NE_L (MJ/kg DM): 7.5. [b]Fatty acid composition (mg/100 g DM): C14:0, 14.76; C16:0, 1871.52; C16:1 c9, 18.50; C18:0, 1197.80; C18:1 c9, 6512.48; C18:2 c9c12, 5471.28; C20:0, 46.73; C18:3 c9c12c15, 19661.66. [c]Calculated as 1000-(NDF+CP+EE+ash)

Table 2: Daily intake (g/head/day) of dietary individual fatty acids by mid-lactating Saanen goats fed a TMR unsupplemented or supplemented with 3 g/100 g DM extruded linseed

	Dietary treatment	
Treatments	C	EL
C12:0	0.020	0.020
C14:0	0.158	0.168
C16:0	12.965	14.275
C16:1 c9	0.149	0.162
C18:0	1.924	2.762
C18:1 c9 (OA)	16.485	21.043
C18:1 c11	0.942	0.942
C18:2 c9c12 (LA)	41.301	45.131
C18:3 c9c12c15 (ALA)	7.769	21.532
C20	0.417	0.449
ΣSFA	15.481	17.673
ΣMUFA	17.575	22.147
ΣPUFA	49.070	66.663
TFA	82.126	106.483

DM: Dry Matter; C: Control group; EL: Extruded Linseed group; OA: Oleic Acid; LA: Linoleic Acid; ALA: α-Linolenic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; TFA: Total Fatty Acids

Table 3: Production performance and gross composition of bulk milk from mid-lactating Saanen goats fed a TMR unsupplemented or supplemented with 3 g/100 g DM extruded linseed

	Dietary treatment			p-values
Diets	C (n = 10)	EL (n = 10)	SEM	DT
Milk yield (kg/group/day)	22.5	24.3	1.40	NS
Milk fat (g/kg)	31.5	34.6	0.75	0.009
Milk protein (g/kg)	33.2	33.8	0.34	NS
Milk lactose (g/kg)	42.9	43.7	0.47	NS

DM: Dry Matter; C: Control group; EL: Extruded Linseed group; SEM: Standard Error of the Mean; DT: Fixed effect of dietary Treatment; NS: Not Significant ($p>0.05$)

by about 24 g per head of which almost all (94%) were C18 FA. Total PUFA, MUFA and SFA intakes of EL group were +1.36, +1.26 and +1.14 fold higher than C group, respectively. The ALA intake was almost 3 fold higher when the diet was supplemented with EL. The estimated intakes of stearic (C18:0) and oleic acids were about +1.44 and +1.28 fold higher in the EL group if compared to control. The EL treatment also slightly increased the intakes of myristic, palmitic, palmitoleic (C16:1 c9), linoleic, and arachidic (C20:0) acids (about +6 to 10%) while no differences were estimated for lauric and cis-vaccenic (C18:1 c11) acids intakes between groups.

Milk yield and gross composition: Results on milk production and milk gross composition are presented in Table 3. The dietary supplementation with EL enhanced milk fat content (+3.1 g kg^{-1}, $p\leq0.01$) while milk yield and milk protein and lactose contents remained unaffected ($p>0.05$).

Milk and cheese fatty acid composition: The EL supplementation significantly affected the FA composition of bulk milk, fresh cheese and 30 days ripened cheese (Table 4 and 5). No significant interaction between dietary treatment and dairy product (DT×DP) was observed for any of the detected variables, therefore the FA profile of milk, fresh and ripened cheeses showed comparable responses to the EL supplementation.

Among SFA, a highly significant reduction in the concentration of palmitic acid (-5.5%, $p\leq0.001$) and a tendency towards lower concentration for myristic acid (-4.6%, $p = 0.072$) were observed in milk of the EL group. Other SFA were instead enhanced by the lipid supplementation, namely caprylic -C8:0-(+7.2%, $p\leq0.01$), capric-C10:0-(+10.5%, $p\leq0.01$), stearic (+21.2%, $p\leq0.001$) and behenic -C22:0-(+25.0%, $p\leq0.001$) acids. No significant differences between groups were observed for caproic (C6:0), lauric and arachidic acids. Overall, total SFA were not significantly affected by dietary treatment. However, HSFA (calculated as: C12:0+4 * C14:0+C16:0) was significantly reduced ($p\leq0.01$) by EL addition to the diet. Milk stearic acid was significantly and positively correlated with milk fat content (Pearson's correlation coefficient equal to 0.77; $p\leq0.001$).

Odd and Branched-Chain Fatty Acids (OCFA and BCFA) generally showed diminished or unchanged responses to dietary EL supplementation. Both total odd-iso and even-iso BCFA were significantly lower in the EL milk than control (-5.3 and -8.6%, respectively; $p\leq0.05$). In particular, significant reductions were observed for C16 iso (-8.3%, $p\leq0.05$), C17 iso (-5.7%, $p\leq0.05$) and a tendency ($0.05<p\leq0.10$) towards lower concentrations was also observed for C14 iso and C15 iso. Total aiso BCFA and total linear OCFA were instead unaffected by dietary treatment ($p>0.10$).

Almost all detected trans-octadecenoic isomers of EL milk and cheeses showed notable increases if compared to control [C18:1 t5: +15.4%, $p\leq0.001$; C18:1 t12-14 (+c6-8): +70.5%, $p\leq0.001$; C18:1 (c14+)t16: +38.5%, $p\leq0.001$]. The sum of t6 to t11 C18:1 (which can be mainly ascribed to VA) also slightly increased (+5.7%) but the difference between C and EL milk was not statistically significant. The raise in the total C18:1 trans was equal to 18.7% ($p\leq0.01$). Lower concentrations with EL supplementation were instead observed for detected trans MUFA with a carbon chain length other than 18 (C16:1 t: -20.0%, $p\leq0.05$; C17:1 t: -20.0%, $p\leq0.05$; C20:1 t: -10.0%, $p\leq0.01$). All MUFA with a cis-9 double bond significantly decreased with EL inclusion in the diet (C10:1 c9: -8.0%, $p\leq0.05$; C14:1 c9: -25.0%, $p\leq0.001$; C16:1 c9: -20.5%, $p\leq0.001$; C17:1 c9: -20.0%, $p\leq0.01$; C18:1 c9: -8.2%, $p\leq0.01$; C20:1 c9: -25.0%, $p\leq0.001$). On a whole, total MUFA were significantly lower (-5.7%, $p\leq0.05$) in milk and cheeses obtained from the goats supplemented with EL.

Total PUFA were not altered by dietary treatment ($p>0.10$). The majority of non-conjugated octadecadienoic acids [both Methylene Interrupted (MID) and Non-Methylene Interrupted (NMID) dienes)] significantly increased (or at least tended to increase) with the lipid supplementation [C18:2 (t, t-NMID+) t9t12: +37.5%, $p\leq0.001$; C18:2 c9t13+t8c12: +55.6%, $p\leq0.001$; C18:2 c, c-MID+t8c13: +11.1%, $p = 0.057$; C18:2 t11c15: +33.3, $p\leq0.001$; C18:2 t9c12: +50.0%, $p\leq0.001$]. Exceptions regarded C18:2 c9t12 and C18:2 c9c12, the former being unaffected ($p>0.10$) and the latter being reduced (-6.6%, $p\leq0.01$) with EL inclusion in the diet. Variable responses were instead observed for detected conjugated octadecadienoic acids (Conjugated Linoleic Acid isomers, CLA) being the sum of C18:2 t11c13 and c9c11 increased (+22.2%; $p\leq0.001$), the sum of C18:2 c9t11, t7c9 and t8c10 decreased (-15.8% $p\leq0.001$) while C18:2 t10c12 and t9t11 were unchanged when EL was added to the diet.

Table 4: Groups of fatty acids (g/100 g of total fatty acids) in bulk milk, fresh cheese and 30 days ripened cheese obtained from mid-lactating Saanen goats fed a TMR unsupplemented or supplemented with 3 g/100 g DM extruded linseed

	Dietary treatment		Dairy product				p-values[a]		Correlations			
									M vs. FC		M vs. RC	
Treatments	C (n = 30)	EL (n = 30)	M (n = 20)	FC (n = 20)	RC (n = 20)	SEM	DT	DP	r[b]	p-values	r	p-values
Σ short chain[c]	12.990	14.030	13.550	13.590	13.41	0.4370	0.005	NS	0.993	<0.001	0.916	<0.001
Σ medium chain[d]	41.980	39.960	40.890	40.900	41.11	0.5880	<0.001	NS	0.998	<0.001	0.989	<0.001
Σ long chain[e]	45.030	46.010	45.560	45.520	45.49	0.9790	NS	NS	0.996	<0.001	0.992	<0.001
Σ saturated[f]	66.710	68.360	67.480	67.450	67.66	0.9000	0.029	NS	0.997	<0.001	0.989	<0.001
Σ odd iso branched chain[g]	0.570	0.540	0.550	0.560	0.55	0.0170	0.017	NS	0.961	<0.001	0.959	<0.001
Σ even iso branched chain[h]	0.350	0.320	0.330	0.330	0.33	0.0170	0.044	NS	0.996	<0.001	0.993	<0.001
Σ aiso branched chain[i]	0.930	0.930	0.930	0.920	0.95	0.0210	NS	NS	0.818	<0.001	0.745	<0.001
HSFA[j]	65.980	62.930	64.280	64.380	64.71	1.2930	0.006	NS	0.997	<0.001	0.994	<0.001
Σ monounsaturated[k]	27.940	26.340	27.240	27.190	26.99	0.7920	0.017	NS	0.998	<0.001	0.989	<0.001
Σ C18:1[l]	26.560	25.170	25.960	25.920	25.72	0.7630	0.031	NS	0.998	<0.001	0.989	<0.001
Σ C18:1 trans[m]	2.19	2.600	2.390	2.400	2.40	0.1620	0.003	NS	0.987	<0.001	0.986	<0.001
Σ trans monounsaturated[n]	2.320	2.720	2.510	2.520	2.53	0.1620	0.004	NS	0.988	<0.001	0.986	<0.001
Σ trans monounsaturated-Σ C18:1 trans[o]	0.140	0.120	0.130	0.120	0.13	0.0060	<0.001	NS	0.778	<0.001	0.787	<0.001
Σ polyunsaturated[p]	5.360	5.300	5.290	5.360	5.35	0.1450	NS	NS	0.963	<0.001	0.965	<0.001
Σ C18:2[q]	4.540	4.350	4.420	4.460	4.45	0.1310	0.090	NS	0.976	<0.001	0.978	<0.001
Σ C18:2 trans[r]	1.200	1.240	1.210	1.230	1.23	0.0740	NS	NS	0.990	<0.001	0.988	<0.001
Σ CLA[s]	0.590	0.490	0.530	0.540	0.54	0.0320	0.001	NS	0.971	<0.001	0.973	<0.001
Σ trans without CLA[t]	3.200	3.820	3.470	3.540	3.53	0.2220	0.001	NS	0.991	<0.001	0.989	<0.001
Σ n3 FA[u]	0.720	0.920	0.800	0.830	0.83	0.0300	<0.001	NS	0.976	<0.001	0.969	<0.001
Σ n6 FA[v]	4.550	4.720	4.590	4.660	4.66	0.1450	NS	NS	0.981	<0.001	0.960	<0.001
n6/n3	7.570	6.280	6.740	6.900	7.12	0.2330	<0.001	NS	0.960	<0.001	0.975	<0.001
Σ unsaturated[w]	33.290	31.650	32.520	32.550	32.34	0.9000	0.029	NS	0.997	<0.001	0.989	<0.001
C18:3 c9c12c15/ C18:2 c9c12 (ALA/LA)	0.160	0.240	0.190	0.200	0.21	0.0080	<0.001	NS	0.993	<0.001	0.990	<0.001
DI_{14}[x]	0.011	0.009	0.009	0.010	0.010	0.0005	<0.001	NS	0.764	<0.001	0.723	<0.001
DI_{16}	0.020	0.017	0.018	0.019	0.019	0.0012	0.001	NS	0.919	<0.001	0.962	<0.001
DI_{17}	0.480	0.400	0.440	0.440	0.44	0.0240	<0.001	NS	0.931	<0.001	0.961	<0.001
DI_{18}	2.440	1.870	2.100	2.170	2.19	0.1140	<0.001	NS	0.992	<0.001	0.990	<0.001

DM: Dry Matter; C: Control group; EL: Extruded Linseed group; M: Milk; FC: Fresh Cheese; RC: Ripened Cheese; SEM: Standard Error of the Mean; DT: Fixed effect of Dietary Treatment; DP: Fixed effect of Dairy Product; CLA: Conjugated Linoleic Acid; FA: Fatty Acids; HSFA: Hypercholesterolemic Saturated Fatty Acids; DI: Desaturase Index. [a]The effect of interaction between dietary treatment and dairy product (DT×DP) was not significant therefore significance is only presented for DT and DP. The p-value is shown if thus being not significant, it shows a tendency ($0.05<p\leq0.10$); NS: Not Significant ($p>0.10$). [b]Pearson's correlation coefficient. [c]C6:0, C7:0, C8:0, C10:0, C10:1 c9. [d]C12:0, C13 iso, C13 aiso, C12:1 c+C13:0, C14 iso, C14:0, C15 iso, C15 aiso, C14:1 c9, C15:0, C16 iso, C16:0, C17 iso, C16:1 t, C17 aiso, C16:1 c9. [e]C17:0, C18 iso, C17:1 t, C17:1 c9, C18:0, Σ C18:1 c9, Σ C18:2, C20:0, C20:1 t, C18:3 c6c9c12, C20:1 c5, C20:1 c9, C20:1 c11, C18:3 c9c12c15, C20:2 c, c n6, C22:0, C20:3 n6, C20:3 n3, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA), C22:6 n3 (DHA). [f]C6:0, C7:0, C8:0, C10:0, C12:0, Σ branched chain, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0. [g]C13 iso, C15 iso, C17 iso. hC14 iso, C16 iso, C18 iso. iC13 aiso, C15 aiso, C17 aiso. [j]Calculated as C12+4*C14+C16. [k]C10:1 c9, C12:1 c+C13:0, C14:1 c9, C16:1 t, C16:1 c9, C17:1 t, C17:1 c9, Σ C18:1, C20:1 t, C20:1 c5, C20:1 c9, C20:1 c11. lC18:1 t5, t6-11, t12-14+c6-8, c9, c11, c12, c14+t16. [m]C18:1 t5, t6-11, t12-14+c6-8. [n]C16:1 t, C17:1 t, Σ C18:1 t, C20:1 t. [o]C16:1 t, C17:1 t, C20:1 t. [p]Σ C18:2, C18:3 c6c9c12, C18:3 c9c12c15, C20:2 c,c n6, C20:3 n6, C20:3 n3, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA), C22:6 n3 (DHA). [q]C18:2 t,t-NMID+t9t12, c9t13+t8c12, c9t12, c,c-MID+t8c13, t11c15, t9c12, c9c12, c9t11+t7c9+t8c10, t10c12, t11c13+c9c11, t9t11. [r]C18:2 t,t-NMID+t9t12, c9t13+t8c12, c9t12, c,c-MID+t8c13, t11c15, t9c12, c9t11+t7c9+t8c10, t10c12, t11c13+c9c11, t9t11. [s]C18:2 c9t11+t7c9+t8c10, t10c12, t11c13+c9c11, t9t11. [t]C16:1 t, C17:1 t, Σ C18:1 t, Σ C18:2 t (without CLA trans), C20:1 t. [u]C18:2 t11c15, C18:3 c9c12c15, C20:3 n3, C20:5 n3 (EPA), C22:5 n3 (DPA), C22:6 n3n (DHA). [v]C18:1 t12-14+c6-8, C18:1 c12, C18:2 t,t-NMID+t9t12, C18:2 c9t12, C18:2 t9c12, C18:2 c9c12, C18:3 c6c9c12, C20:2 c,c n6, C20:3 n6, C20:4 n6 (AA). [w]C10:1 c9, C12:1 c+C13:0, C14:1 c9, C16:1 t, C16:1 c9, C17:1 t, C17:1 c9, Σ C18:1, Σ C18:2, C20:1 t, C18:3 c6c9c12, C20:1 c5, C20:1 c9, C20:1 c11, C18:3 c9c12c15, C18:2 c9t11+t7c9+t8c10, C18:2 t10c12, C18:2 t11c13+c9c11, C18:2 t9t11, C20:2 c,c n6, C20:3 n6, C20:3 n3, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA), C22:6 n3 (DHA). [x]Desaturase indexes calculated as: DI_{14} = C14:1 c9/C14:0; DI_{16} = C16:1 c9/C16:0; DI_{17} = C17:1 c9/C17:0; DI_{18} = C18:1 c9/C18:0

The concentration of total n3 FA in milk was raised by about 28% in the EL group if compared to control ($p\leq0.001$). Such increase was solely attributable to α-linolenic and eicosatrienoic (C20:3 c11c14c17) acids as the dietary treatment did not show any significant influence on very long-chain n3 PUFA (eicosapentaenoic -C20:5 c5c8c11c14c17-EPA, docosapentaenoic-C22:5 c7c10c13c16c19-DPA and docosahexahenoic-C22:6 c4c7c10c13c16c19-DHA-acids). The ratio between ALA and LA in goat milk fat sharply increased with dietary inclusion of EL ($p\leq0.001$). Detected long-chain n6 FA deriving from LA metabolism (γ-linolenic-C18:3 c6c9c12-GLA, dihomo-γ-linolenic-C20:3c8c11c14-DGLA and arachidonic-C20:4 c5, c8, c11, c14-AA, acids) were lower in the milk obtained from the EL-supplemented goats. As a consequence of both n3 and n6 FA

Table 5: Individual fatty acids (g/100 g of total fatty acids) in bulk milk, fresh cheese and 30 days ripened cheese obtained from mid-lactating Saanen goats fed a TMR unsupplemented or supplemented with 3 g/100 g DM extruded linseed

	Dietary treatment		Dairy product[a]				p-values[b]		Correlations			
									M vs. FC		M vs. RC	
Treatments	C (n = 30)	EL (n = 30)	M (n = 20)	FC (n = 20)	RC (n = 20)	SEM	DT	DP	r[c]	p-values	r	p-values
C6:0	2.160	2.190	2.160	2.180	2.170	0.0430	NS	NS	0.851	<0.001	0.861	<0.001
C7:0	0.020	0.030	0.020	0.030	0.030	0.0020	0.079	NS	0.756	<0.001	0.835	<0.001
C8:0	2.490	2.670	2.590	2.580	2.560	0.0740	0.005	NS	0.991	<0.001	0.993	<0.001
C10:0	8.080	8.930	8.540	8.560	8.410	0.3260	0.002	NS	0.997	<0.001	0.842	<0.001
C10:1 c9	0.250	0.230	0.240	0.240	0.240	0.0100	0.020	NS	0.958	<0.001	0.972	<0.001
C12:0	3.280	3.320	3.300	3.310	3.290	0.1530	NS	NS	0.997	<0.001	0.949	<0.001
C13 iso	0.020	0.020	0.020	0.020	0.020	0.0010	NS	NS	0.564	0.010	0.636	0.003
C13 aiso	0.032	0.027	0.029	0.030	0.030	0.0017	0.002	NS	0.986	<0.001	0.988	<0.001
C12:1 c + C13:0	0.130	0.120	0.130	0.130	0.130	0.0070	NS	NS	0.993	<0.001	0.996	<0.001
C14 iso	0.100	0.090	0.090	0.090	0.090	0.0050	0.098	NS	0.993	<0.001	0.979	<0.001
C14:0	9.160	8.740	8.910	8.940	8.990	0.2770	0.072	NS	0.997	<0.001	0.997	<0.001
C15 iso	0.200	0.180	0.190	0.190	0.190	0.0080	0.084	NS	0.988	<0.001	0.992	<0.001
C15 aiso	0.320	0.320	0.320	0.320	0.320	0.0120	NS	NS	0.992	<0.001	0.995	<0.001
C14:1 c9	0.080	0.060	0.070	0.070	0.070	0.0050	<0.001	NS	0.851	<0.001	0.840	<0.001
C15:0	0.920	0.890	0.900	0.900	0.910	0.0260	NS	NS	0.986	<0.001	0.993	<0.001
C16 iso	0.240	0.220	0.230	0.230	0.230	0.0120	0.028	NS	0.993	<0.001	0.992	<0.001
C16:0	26.080	24.650	25.330	25.310	25.460	0.3630	<0.001	NS	0.998	<0.001	0.989	<0.001
C17 iso	0.350	0.330	0.340	0.340	0.340	0.0110	0.011	NS	0.932	<0.001	0.929	<0.001
C16:1 t	0.050	0.040	0.050	0.050	0.050	0.0050	0.011	NS	0.808	<0.001	0.730	<0.001
C17 aiso	0.580	0.590	0.580	0.570	0.600	0.0140	NS	NS	0.579	0.007	0.331	NS
C16:1 c9	0.440	0.350	0.400	0.390	0.390	0.0210	<0.001	NS	0.934	<0.001	0.968	<0.001
C17:0	0.600	0.590	0.590	0.590	0.590	0.0240	NS	NS	0.965	<0.001	0.963	<0.001
C18 iso	0.010	0.010	0.010	0.010	0.010	0.0004	NS	NS	0.757	<0.001	0.633	0.003
C17:1 t	0.050	0.040	0.050	0.050	0.050	0.0040	0.045	NS	0.986	<0.001	0.986	<0.001
C17:1 c9	0.250	0.200	0.220	0.220	0.230	0.0190	0.008	NS	0.969	<0.001	0.982	<0.001
C18:0	11.680	14.160	12.920	12.830	13.000	0.4660	<0.001	NS	0.996	<0.001	0.994	<0.001
C18:1 t5	0.013	0.015	0.013	0.015	0.014	0.0008	<0.001	NS	0.371	0.090	0.300	NS
C18:1 t6-11	1.740	1.840	1.800	1.780	1.780	0.1050	NS	NS	0.994	<0.001	0.986	<0.001
C18:1 t12-14 + c6-8	0.440	0.750	0.570	0.600	0.600	0.0650	<0.001	NS	0.934	<0.001	0.931	<0.001
C18:1 c9 (OA)	23.470	21.550	22.660	22.520	22.340	0.7450	0.003	NS	0.997	<0.001	0.994	<0.001
C18:1 c11	0.390	0.360	0.360	0.390	0.380	0.0170	0.033	NS	0.126	NS	0.257	NS
C18:1 c12	0.250	0.300	0.270	0.280	0.280	0.0200	0.002	NS	0.948	<0.001	0.941	<0.001
C18:1 c14 + t16	0.260	0.360	0.280	0.330	0.320	0.0240	<0.001	NS	0.897	<0.001	0.913	<0.001
C19:0	0.080	0.080	0.080	0.080	0.080	0.0040	NS	NS	0.634	0.003	0.535	0.015
C18:2 t, t-NMID + t9t12	0.080	0.110	0.090	0.100	0.100	0.0110	<0.001	NS	0.920	<0.001	0.903	<0.001
C18:2 c9t13 + t8c12	0.009	0.014	0.013	0.011	0.011	0.0009	<0.001	NS	0.889	<0.001	0.848	<0.001
C18:2 c9t12	0.210	0.230	0.220	0.220	0.220	0.0180	NS	NS	0.998	<0.001	0.996	<0.001
C18:2 c, c-MID + t8c13	0.180	0.200	0.190	0.190	0.200	0.0130	0.057	NS	0.994	<0.001	0.993	<0.001
C18:2 t11c15	0.120	0.160	0.140	0.140	0.140	0.0060	<0.001	NS	0.948	<0.001	0.920	<0.001
C18:2 t9c12	0.020	0.030	0.020	0.020	0.020	0.0020	<0.001	NS	0.561	0.010	0.592	0.006
C18:2 c9c12 (LA)	3.340	3.120	3.210	3.240	3.230	0.0830	0.002	NS	0.975	<0.001	0.980	<0.001
C20:0	0.280	0.290	0.290	0.290	0.290	0.0070	NS	NS	0.987	<0.001	0.972	<0.001
C20:1 t	0.030	0.027	0.027	0.030	0.029	0.0015	0.004	NS	0.855	<0.001	0.851	<0.001
C18:3 c6c9c12 (GLA)	0.006	0.005	0.006	0.005	0.005	0.0005	0.052	NS	-0.034	NS	-0.318	NS
C20:1 c5	0.002	0.001	0.002	0.001	0.001	0.0002	0.014	NS	0.432	0.057	0.455	0.044
C20:1 c9	0.040	0.030	0.040	0.040	0.040	0.0010	<0.001	NS	0.505	0.023	0.906	<0.001
C20:1 c11	0.063	0.060	0.061	0.061	0.062	0.0018	0.040	NS	0.679	0.001	0.970	<0.001
C18:3 c9c12c15 (ALA)	0.450	0.620	0.530	0.540	0.530	0.0220	<0.001	NS	0.985	<0.001	0.982	<0.001
CLA c9t11 + t7c9 + t8c10	0.570	0.480	0.510	0.530	0.530	0.0320	<0.001	NS	0.975	<0.001	0.975	<0.001
CLA t10c12	0.001	0.001	0.001	0.001	0.001	0.0002	NS	NS	0.519	0.019	0.736	<0.001
CLA t11c13 + c9c11	0.009	0.011	0.010	0.010	0.010	0.0008	<0.001	NS	0.614	0.004	0.912	<0.001
CLA t9t11	0.003	0.003	0.004[a]	0.003[ab]	0.002[b]	0.0005	NS	0.015	0.218	NS	0.497	0.026
C20:2 c, c n6	0.020	0.020	0.020	0.020	0.020	0.0010	NS	NS	0.605	0.005	0.803	<0.001
C22:0	0.040	0.050	0.040	0.040	0.040	0.0020	<0.001	NS	0.944	<0.001	0.937	<0.001
C20:3 n6 (DGLA)	0.021	0.020	0.020	0.020	0.021	0.0011	0.038	NS	0.747	<0.001	0.881	<0.001
C20:3 n3	0.004	0.005	0.005	0.004	0.004	0.0004	0.001	NS	0.435	0.055	0.391	0.088
C20:4 n6 (AA)	0.170	0.150	0.150	0.160	0.160	0.0080	<0.001	NS	0.959	<0.001	0.967	<0.001
C20:5 n3 (EPA)	0.030	0.030	0.030	0.030	0.030	0.0010	NS	NS	0.648	0.002	0.692	0.001
C22:5 n3 (DPA)	0.090	0.090	0.080	0.090	0.090	0.0040	NS	NS	0.871	<0.001	0.924	<0.001
C22:6 n3 (DHA)	0.020	0.020	0.020	0.020	0.020	0.0010	NS	NS	0.477	0.034	0.082	NS

DM: Dry Matter; C: Control group; EL: Extruded Linseed group; M: Milk; FC: Fresh Cheese; RC: Ripened Cheese; SEM: Standard Error of Mean; DT: fixed effect of Dietary Treatment; DP: fixed effect of Dairy Product; CLA: Conjugated Linoleic Acid; t: trans; c: cis; OA: Oleic Acid; NMID: Non Methylene Interrupted Diene; MID: Methylene Interrupted Diene; LA: Linoleic Acid; GLA: Gamma-Linolenic Acid; ALA: Alpha-Linolenic Acid; DGLA: Dihomo-Gamma-Linolenic Acid; AA: Arachidonic Acid; EPA: Eicosapentaenoic Acid; DPA: Docosapentaenoic Acid; DHA: Docosahexaenoic Acid. [a]Means within a row with different superscripts differ significantly. [b]The effect of interaction between Dietary Treatment and Dairy Product (DT×DP) was not significant therefore significance is only presented for DT and DP. The p-value is shown if thus being not significant, it shows a tendency ($0.05<p\leq0.10$); NS: Not Significant ($p>0.10$). [c]Pearson's correlation coefficient

responses, the EL supplementation substantially diminished the n6/n3 fatty acids ratio of milk (-17.0%; $p \leq 0.001$).

Mammary Δ9-desaturase activity, estimated by the computation of different Desaturase Indexes (DI, ratios of Δ9-desaturase products and precursors, i.e., DI_{14}: C14:1 c9/C14:0, DI_{16}: C16:1 c9/C16:0, DI_{17}: C17:1 c9/C17: 0, DI_{18}: 18:1 c9/C18:0) was significantly lower ($p \leq 0.001$) in the goats fed the EL diet if compared to control.

The effect of cheese-making and ripening was negligible on FA composition and no significant differences were found among milk, fresh and ripened cheeses apart from CLA t9t11 which was significantly lower in the ripened cheese if compared to milk.

Highly significant and positive correlations were found among the concentrations of FA in milk and cheeses (Table 4 and 5).

DISCUSSION

Milk yield and gross composition: The results of the current study confirm that the addition of linseed (in various forms such as linseed oil, whole linseed, extruded linseed and extruded linseed cake) to dairy goats' diets does not significantly influence milk yield response from mid or late-lactating goats (Bernard *et al*., 2009b; Dehareng *et al*., 2009; Li *et al*., 2012; Marin *et al*., 2011; Nudda *et al*., 2006). However, the use of quite high amount of formaldehyde-treated linseed was reported to significantly lessen milk yield in mid-lactating goats (Bernard *et al*., 2005).

Regarding milk fat, linseed supplementation was already reported to enhance its content when used as oil (Bernard *et al*., 2009b; Chilliard *et al*., 2006; Li *et al*., 2012; Marin *et al*., 2011) or in rumen protected form (Bernard *et al*., 2005). However, other researchers showed no significant changes in milk fat content as a consequence of linseed addition to the diet when used as whole or extruded seeds (Dehareng *et al*., 2009) or as cakes (Nudda *et al*., 2006). In this study, the observed noticeable increase with EL supplementation occurred despite the low F:C ratio (40:60) of the experimental TMR. Such increase could be attributed to; the nutritional regulation of lipogenic gene expression, abundance and/or activity and the higher dietary FA supply (Table 2) brought to the mammary gland for milk fat synthesis. The results of the current trial also confirm that dairy goats do not usually suffer from diet-induced milk fat depression and regardless of lactation stage, they even commonly respond to lipid supplementation by raising milk fat content sharply (Chilliard *et al*., 2007). The quantitative response obtained on milk fat content in the current study (+3.1 g kg^{-1}) fell within the range (+1.9 to +7.2 g kg^{-1}) obtained in other published trials (Bernard *et al*., 2009b, 2005; Chilliard and Ferlay, 2004; Dehareng *et al*., 2009; Li *et al*., 2012; Marin *et al*., 2011). Quantitative differences among experimental studies have to be attributed to many different interacting factors both animal-based ones (such as the lactation stage and the productive potential of the goats) and dietary-related ones (amount of linseed inclusion, composition of the basal diet and total fat content of the diet).

The lack of significant influence of EL supplementation on milk protein content observed in the current study is consistent with the majority of published trials (Bernard *et al*., 2009a; Dehareng *et al*., 2009; Li *et al*., 2012; Marin *et al*., 2011; Nudda *et al*., 2006). Some studies reported significant but slight (+0.8 to 2.5 g kg^{-1}) if compared to milk fat, increases in milk protein after adding linseed oil, rumen protected or rumen unprotected linseed to the diet (Bernard *et al*., 2009b, 2005; Chilliard *et al*., 2003a).

Milk from mid-lactating goats fed high concentrate diets can suffer from reversion in the fat to protein ratio, resulting in negative alterations of the coagulation properties and in lowered cheese quality (Morand-Fehr *et al*., 2007). From the results of the current trial, it can be stated that dietary supplementation of EL prevents from fat/protein percentage-reversion risk thus allowing a better control of cheese processing. This aspect is of particular importance in Mediterranean countries where goat milk is mainly used for cheese-making.

Till now, only few studies (all of them using linseed oil) evaluated the effect of linseed supplementation on milk lactose response in dairy goats. As, already reported by Li *et al*. (2012) and Marin *et al*. (2011) also in this study, EL addition to the diet did not determine a significant variation in the lactose content. Such response could derive from comparable glucose availability for lactose synthesis within the mammary gland between EL and C groups as the experimental diets were characterized by comparable NE_L.

Milk and cheese fatty acid composition: Previous studies aimed to evaluate the effects of pure EL-supplemented diets on FA composition of goat milk and derived products were conducted on early lactating animals (Battacone *et al*., 2007) or groups of animals at different lactation stages (Dehareng *et al*., 2009). In early lactation, the FA composition of goat milk may vary considerably while in mid and late lactation it tends instead to stabilize (Chilliard *et al*., 2003a). In the current trial, the goats were in mid-lactation at the beginning of the experiment which

can reasonably allow to exclude a confounding effect due to lactation stage on milk FA composition and to attribute the observed variations to feeding aspects only.

Δ9-desaturase activity: The Δ9-desaturase enzyme system, namely the stearoyl-CoA desaturase (SCD, EC 1.14.19.1), plays a key role in lipogenesis being responsible for Δ9-desaturation (addition of a cis double bond at the Δ9, 10 position) of methylene-interrupted fatty acyl-CoA substrates (Paton and Ntambi, 2009). Among various desaturase indexes, DI_{14} is commonly considered the best indicator of mammary Δ9-desaturase activity because almost all myristoleic acid synthesis from myristic acid is regulated in the mammary gland by the activity of this enzyme (Shingfield *et al*., 2010). In the current trial, no distinction among t6 to t10 octadecenoic isomers and CLA isomers c9t11, t7c9 and t8c10 could be made, therefore, DI_{CLA} (C18:2 c9t11/C18:1 t11) was not calculated. All other calculated Δ9-desaturase ratios (DI_{14}, DI_{16}, DI_{17} and DI_{18}) significantly declined with dietary addition of EL, confirming the data obtained in the majority of previously published trials on goats (Bernard *et al*., 2009a, 2005; Chilliard *et al*., 2004; Marin *et al*., 2011). On the whole, such results are consistent with the significant declines in measured mammary Δ9-desaturase activity observed during *in vitro* incubation of goat mammary tissue with ALA (Bickerstaffe and Annison, 1970) or during *in vivo* studies as a consequence of dietary inclusion of linseed (Bernard *et al*., 2005, 2009a). The observed influence of dietary EL supplementation on goat mammary Δ9-desaturase activity may be due to inhibitory effects exerted by dietary ALA escaping from the rumen and/or by trans intermediate octadecatrienoic, octadecadienoic and octadecenoic acids formed during ruminal BH of dietary ALA (Bernard *et al*., 2008; Chilliard *et al*., 2007).

Saturated fatty acids: In goats, short-chain SFA concentrations were reported to be either unchanged (Battacone *et al*., 2007; Bernard *et al*., 2009b; Chilliard and Ferlay, 2004; Marin *et al*., 2011; Nudda *et al*., 2006), reduced (Bernard *et al*., 2009b, 2005) or increased (Bernard *et al*., 2009a; Marin *et al*., 2013) with dietary addition of linseed. On the contrary, medium-chain SFA (particularly myristic and palmitic acids) significantly declined in almost all published studies (Battacone *et al*., 2007; Bernard *et al*., 2009a, b, 2005; Li *et al*., 2012; Marin *et al*., 2011; Nudda *et al*., 2006). These results were also confirmed in the current trial where unchanged or even positive responses were found for FA with <12 carbon atoms while myristic and palmitic acids declined with dietary EL supplementation. Milk short and medium-chain SFA from 6-14 carbon atoms are entirely synthesised *de novo* within the mammary gland thanks to the activity of Acetyl-CoA Carboxylase (ACC, E.C. 6.4.1.2) and Fatty Acid Synthase (FAS, E.C. 2.3.1.85). The 40% of palmitic acid is also synthesised this way while the remaining part is absorbed from circulation (Chilliard and Ferlay, 2004). Declines in goat milk concentration of *de novo* synthesised FA were not associated with repression (due to inhibitory effects exerted by dietary and ruminally-derived long-chain unsaturated fatty acids-UFA-absorbed in the small intestine) of goat mammary ACC and FAS mRNA abundance and/or activity (Bernard *et al*., 2009a, 2008). This suggests that the goat regulation of mammary lipogenesis in response to vegetable lipid supplementation may be related to factors other than altered mammary gene expression or lipogenic enzyme activities (Bernard *et al*., 2009a). The peculiar response of the goat to plant lipid supplementation (less marked or even opposite response of short-chain if compared to medium-chain SFA) may be ascribed to the fact that short-chain SFA partially origin from metabolic pathways that do not involve malonyl-CoA and ACC activity (Chilliard *et al*., 2003a). Regarding palmitic acid as its daily dietary ingestion was slightly higher in the EL group (Table 2), the observed reduction in EL milk should be mainly the consequence of a decreased synthesis within the mammary gland. The peculiar response of goats to EL supplementation in the short and medium-chain SFA synthesis is very interesting for a human health perspective. Caproic, caprylic and capric acids have been used for the treatment of malabsorption syndromes, cardiovascular diseases, pancreatic and intestinal disorders and problems related to undernourishment and premature infant nutrition (Sanz-Sampelayo *et al*., 2007). On the contrary, lauric, myristic and palmitic acids are well known to exert negative (hypercholesterolaemic) health effects in humans when consumed in excess (Parodi, 2009).

Odd and branched chain FA are interesting for their ability to predict rumen function (Fievez *et al*., 2012) and to possess anticancer properties [mainly ascribed to 13-methyltetradecanoic (C15 iso) and 14-methylpentadecanoic (C16 iso) acids] (Parodi, 2009). These compounds derive from microbial synthesis which is known to be very active in the caprine species as a consequence, goat milk fat is particularly enriched in OBCFA (Sanz-Sampelayo *et al*., 2007). Despite that till now only few studies evaluated the effects of linseed supplemented diets on these FA in goat milk fat. If considering individual OBCFA, various responses have been reported by different researchers. However, the general tendency is to decline or at least to remain

unaffected by linseed supplementation (Bernard *et al*., 2009b, 2005; Marin *et al*., 2011). The responses observed in the current study (lowered total iso BCFA and unchanged total aiso BCFA and linear OCFA with dietary addition of EL) may be mainly ascribed to modifications in the relative abundance of specific bacterial populations within the rumen (Vlaeminck *et al*., 2006). Among rumen bacteria, cellulolytic contain high amounts of iso BCFA (with only few species particularly enriched in aiso forms) while amylolytic are relatively enriched in linear OCFA. The growth of amylolytic strains is known to be less affected than that of cellulolytic ones by the inhibitory effects of long-chain FA (and therefore by dietary lipid supplementation) (Vlaeminck *et al*., 2006) which could explain the different response observed between linear OCFA and iso BCFA in the current study. Another possible explanation is that linear OCFA are partially synthesised *de novo* from propionate in the mammary gland (Vlaeminck *et al*., 2006). Dietary addition of vegetable lipids was reported to determine a shift in rumen fermentation towards higher propionate production (Andres *et al*., 2011; Li *et al*., 2009). Therefore, the inhibitory effects of long-chain FA on rumen bacteria may also have been counter balanced by increased endogenous synthesis of linear OCFA determined by an increase in their precursor supply.

The increase of stearic acid in milk fat due to dietary addition of linseed is a response frequently reported in goats (Bernard *et al*., 2009a, b, 2005; Chilliard *et al*., 2007; Li *et al*., 2012; Marin *et al*., 2011; Nudda *et al*., 2006). In the current study, such raise was the consequence of the higher dietary stearic acid and C18 UFA supplies and intakes (Table 1 and 2), the latter FA being extensively biohydrogenated to stearic acid by the rumen microflora (Chilliard and Ferlay, 2004). The observed lack of significant differences in milk total PUFA and total trans-octadecadienoic acids and the contemporary notable increases in total trans-octadecenoic acids and stearic acid, suggest that the BH rate of dietary UFA was probably high and that the release of lipids from linseed was probably slow.

The highly significant and positive correlation observed between stearic acid and milk fat content in the current study was also previously found by Chilliard *et al*. (2003b) and confirms the role of stearic acid as a major regulating factor of mammary fat yield in goats (Bernard *et al*., 2008; Sanz-Sampelayo *et al*., 2007).

Monounsaturated fatty acids: More than 50% of all the OA secreted in milk is synthesised endogenously within the mammary gland from stearic acid through the activity of the Δ9-desaturase enzyme (Chilliard and Ferlay, 2004). In the current study despite the higher dietary ingestion (+4.558 g/head/day) of OA and the higher availability of stearic acid for milk fat synthesis, the concentration of OA in milk from EL-supplemented goats was significantly lower than in milk from the C group. Such response is consistent with the observed notable decrease in the estimated Δ9-desaturase activity within the mammary gland. In other trials, OA in goat milk fat was either found to increase (Bernard *et al*., 2009a, b, 2005; Chilliard *et al*., 2007; Li *et al*., 2012; Nudda *et al*., 2006) or to remain unaffected (Battacone *et al*., 2007; Bernard *et al*., 2009b; Chilliard *et al*., 2007; Dehareng *et al*., 2009; Marin *et al*., 2011) by linseed addition to the diet. Differences among studies may derive from diverse dietary OA and stearic acid supplies from the diets and/or different degrees of Δ9-desaturase inhibition exerted by dietary UFA.

Similarly to what observed for OA, milk concentrations of all other detected MUFA with a cis-9 double bond significantly decreased with the addition of EL in the diet, a result also previously reported by Bernard *et al*. (2009a, b, 2005) and Marin *et al*. (2011). As pointed out for OA such decreases have to be attributed to the decreased Δ9-desaturase activity in mammary secretory cells.

Regarding other detected cis-octadecenoic acids, the observed significant increases in C18:1 *c*12 and C18:1 c14 (+t16) with dietary addition of EL confirm previous findings (Bernard *et al*., 2009b; Marin *et al*., 2011). Such cis-octadecenoic isomers can in fact be formed during the BH of dietary UFA (Shingfield *et al*., 2010), the intake of which was higher in the EL-supplemented if compared to unsupplemented goats.

Linseed addition to the diet was already reported to determine significant increases in trans-octadecenoic isomers in many trials conducted with goats (Bernard *et al*., 2009a, b, 2005; Chilliard *et al*., 2007; Li *et al*., 2012; Marin *et al*., 2011; Nudda *et al*., 2006). Vaccenic and other trans-octadecenoic isomers are intermediate products of UFA BH within the rumen (Shingfield *et al*., 2010) which explains their sharp increase with dietary addition of UFA-rich vegetable lipids including linseed. The raise in milk VA was shown to be of greater extent when grassland hay was used as basal diet instead of maize silage while an opposite trend was observed for t13 and t14 isomers (Bernard *et al*., 2009b). This seems to be consistent with the greater relative extent of increase observed in the current trial in C18:1 t12-14 (+c6-8) if compared to the increase in the sum of t6-11 isomers. Moreover, addition of oil as part of seeds if compared to free forms was found to be less efficient in increasing VA in goat milk fat (Chilliard *et al*., 2003a) which could also partly explain the relatively low increasing response of C18:1 t6-11 isomers obtained in the current trial.

Polyunsaturated fatty acids: Significant increases in goat milk ALA with dietary addition of linseed were already observed by Battacone *et al.* (2007), Bernard *et al.* (2009a, b, 2005), Chilliard *et al.* (2007), Li *et al.* (2012), Marin *et al.* (2011) and Nudda *et al.* (2006). In the current study, the increase of ALA concentration in EL milk was highly statistically significant but limited if compared to that observed in other studies where linseed oil was used (Bernard *et al.*, 2009b; Li *et al.*, 2012; Marin *et al.*, 2011). As previously mentioned, this should be the consequence of a higher BH efficiency of dietary ALA when linseed is supplemented as part of the seeds if compared to the administration of free oil (Chilliard *et al.*, 2003a). The increase in milk ALA concentration in this and other studies (Bernard *et al.*, 2009a, b) was lower if compared to the increases observed for some octadecadienoic and octadecenoic acids. The explanation relies in the BH process of ALA which is firstly isomerised to a number of non-conjugated and partially conjugated C18:3 acids then hydrogenated to non-conjugated and conjugated C18:2 acids, subsequently hydrogenated to cis and trans-C18:1 acids and finally to stearic acid (Lee and Jenkins, 2011; Shingfield *et al.*, 2010).

Very long-chain n3 FA were detected only in few published studies evaluating the effect of linseed supplementation on goat milk FA profile and results consistently differed among trials. Eicosatrienoic acid (C20:3 c11c14c17) was detected only by Marin *et al.* (2011) who showed a lack of significant influence of lipid supplementation. Eicosapentaenoic Acid (EPA, C20:5 c5c8c11c14c17) was reported either to increase (Marin *et al.*, 2011; Nudda *et al.*, 2006), decrease (Bernard *et al.*, 2009b) or remain unaffected (Battacone *et al.*, 2007; Bernard *et al.*, 2009b) by dietary addition of linseed. Docosapentaenoic acid (DPA, C22:5 c7c10c13c16c19) was either reported to remain unaffected (Bernard *et al.*, 2009b; Marin *et al.*, 2011) or decrease (Bernard *et al.*, 2009b) while Docosahexaenoic Acid (DHA, C22:6 c4c7c10c13c16c19) was usually reported to be unchanged (Battacone *et al.*, 2007; Bernard *et al.*, 2009b; Marin *et al.*, 2011; Nudda *et al.*, 2006) with EL supplementation. The different response observed for very long-chain n3 FA if compared to ALA (which is their precursor) is probably due to the fact that the conversion of the latter to the former FA requires the Δ6-desaturase enzyme which is known to be limiting in lactating ruminants (Bernal-Santos *et al.*, 2010).

Linoleic acid concentration in milk was significantly reduced with dietary inclusion of EL despite the higher dietary LA intake than control (+3.83 g/head/day). Decreases (Bernard *et al.*, 2009a, b, 2005; Chilliard and Ferlay, 2004) or unchanged (Li *et al.*, 2012; Marin *et al.*, 2011; Nudda *et al.*, 2006) responses are common observations in goats. The result obtained in the current trial suggests that dietary LA was very largely hydrogenated as already pointed out by other researchers who reported low apparent transfer rate of LA with dietary addition of linseed (Bernard *et al.*, 2005). The results are also consistent with the previously hypothesized high BH efficiency with dietary EL supplementation. For other detected long-chain n6 FA (GLA, DGLA and AA), the observed decline in milk from EL-supplemented goats may be due to lower availability of LA as precursor (Patterson *et al.*, 2012). In addition, as already pointed out by Ebrahimi *et al.* (2013), the existence of competitions between long-chain members of n3 and n6 FA for the same desaturation and elongation enzymes may not be excluded.

The n6/n3 FA ratio is commonly used to assess the nutritional value of lipids for human consumption. A strong imbalance towards high n6 FA intake at the expense of n3 from the diet is positively correlated with a number of widespread human diseases (Simopoulos, 2011). In ruminant derived food products, this ratio is strongly influenced by dietary lipids fed to the animals. Enhancing dietary ALA usually leads to a decrease in the n6/n3 FA ratio (Ferlay *et al.*, 2010; Gomez-Cortes *et al.*, 2009; Marin *et al.*, 2011). In the current trail such decline was ascribed to both increased n3 FA and decreased n6 FA concentrations in milk fat. The sharp increase found in this study in milk ALA/LA ratio, respective precursors of FA of the n3 and n6 families is also a common observation when linseed is added to goats' diet (Chilliard *et al.*, 2007).

Many detected octadecadienoic isomers are known to be intermediate products of ruminal BH of dietary ALA which explains their increase in milk fat from EL-supplemented goats if compared to control in the current study. This regarded, for example, vaccelenic acid (C18:2 t11c15) which is known to be formed after c9 and/or t9 double bond hydrogenation of partially conjugated C18:3 c9t11c15 and t9t11c15 (in turn formed by means of ALA isomerisation at the first step of its BH process (Destaillats *et al.*, 2005). Vaccelenic acid was also found to increase sharply with dietary addition of linseed in many other trials conducted with goats (Bernard *et al.*, 2009b; Chilliard *et al.*, 2007; Marin *et al.*, 2011). The octadecadienoic isomers c9t13 and t8c12 coeluted with the applied chromatographic conditions. While the latter isomer mainly derives from LA BH (Honkanen *et al.*, 2012), the former is known to be formed according to two main metabolic pathways: within the rumen during the biohydrogenation of ALA (hydrogenation of C18:3 c9t13c15) and within the mammary gland, being another

long-chain Δ9-desaturase product (C18:1 t13 as precursor and formed, in turn, during OA and ALA metabolism in the rumen) (Lee and Jenkins, 2011; Shingfield *et al*., 2010). The significantly lower estimated Δ9-desaturase activity found in the current study with dietary addition of EL leads us to hypothesize that endogenous synthesis is only responsible for the formation of a restricted part of milk C18:2 c9t13 while the major part probably derives from ALA metabolism in the rumen. This isomer was already reported to significantly increase in milk from goats (Bernard *et al*., 2009b; Chilliard *et al*., 2004, 2003a, c) with dietary addition of linseed. A tendency towards higher concentration was also observed for C18:2 t8c13 (which in the current trial coeluted with an unidentified cis-cis MID), confirming the results previously found by Marin *et al*. (2011) and supporting the hypothesis that this isomer may derive, at least partly from ruminal BH of dietary ALA.

The lack of significant increasing response observed for C18:2 c9t12 was also reported by Marin *et al*. (2011) and may indicate that this isomer mainly derives from LA metabolism in the rumen (Honkanen *et al*., 2012). Other detected Δ9,12 octadecadienoic isomers (t9c12 and t9t12, the latter coeluting with a non-identified trans-trans NMID in the current trial) are thought to be intermediate products of LA BH (Honkanen *et al*., 2012) but they were found to be significantly higher in milk from EL-supplemented goats in this study and other published trials (Bernard *et al*., 2009b; Marin *et al*., 2011). It should be mentioned that the formation of Δ9, 12 isomers during ALA BH (saturation of non-conjugated C18:3 c9t12c15 and/or c9t12t15) (Loor *et al*., 2004) is plausible and may explain the significant increases found in C18:2 t9c12 and C18:2 t9t12 in the current study and in C18:2 c9t12 in the study published by Bernard *et al*. (2009b) with dietary addition of linseed oil.

Rumenic acid (the most abundant CLA isomer in ruminant dairy fat) has been shown to possess a wide range of positive biological activities including protection against carcinogenesis, diabetes and cardiovascular diseases (Parodi, 2009). With the chromatographic conditions applied in the current trial, RA coeluted with other CLA isomers (C18:2 t7c9 and t8c10). Their sum (likely to be mainly attributed to RA) significantly declined with dietary addition of EL (Table 5). The decrease of RA is in contrast with the results previously found in almost all published trials with dietary addition of linseed. However, Ebrahimi *et al*. (2013) recently reported decreased RA in meat from goats with increasing the amount of linseed oil in the diet which points out in the direction of the results obtained in the current study. Concerning CLA isomers t7c9 and t8c10, they were also reported to rise in milk from linseed supplemented goats (Bernard *et al*., 2009b). The >80% of total milk RA derives from *de novo* synthesis within the mammary gland, mediated by the activity of Δ9-desaturase on VA formed during BH of OA, LA and ALA and escaping complete ruminal BH (Chilliard *et al*., 2003a). The remaining small part of total RA is directly formed within the rumen at the first step of BH of dietary LA (Shingfield *et al*., 2010). CLA t7c9 found in goat milk is most likely another long-chain Δ9-desaturase product. In fact, endogenous synthesis at the expense of C18:1 t7 as precursor has been demonstrated to be the main metabolic pathway of milk CLA t7c9 formation in lactating cows (Shingfield *et al*., 2010). Estimated daily intakes of dietary OA, LA and ALA were higher in the EL-supplemented goats (Table 2). Nevertheless the sum of t6 to t11 octadecenoic isomers even if higher in EL milk was not significantly different between groups. Ruminal BH of UFA released from linseed is probably more efficient if compared to other vegetable lipid supplements (e.g., sunflower) (Chilliard *et al*., 2003a; Marin *et al*., 2013) which were reported to determined very sharp increases in the RA content in milk fat. In this respect, the presentation form of the lipid supplement also plays an important role. As previously mentioned, if compared to direct free oil administration, oil seeds usually lead to higher BH efficiency (which occurs slowly and almost completely), leading to lower transfer of PUFA, trans-FA (including VA) and RA and to higher transfer of stearic acid to milk (Chilliard *et al*., 2003a). It has to be added that for a given lipid supplement, RA in goat milk fat was shown to respond differently according to the type of forage and F:C ratio of the diet. In particular, the response of RA to linseed oil supplementation seems to be of lesser extent when using maize silage than hay or fresh grass and with low F:C ratios of the diet (Chilliard *et al*., 2006) as it occurred in the current trial. Such evidences, combined with the significantly lower estimated Δ9-desaturase activity in the mammary gland, may explain the observed reduction of CLA c9t11+t7c9+t8c10 in EL milk.

CLA t11c13 and c9c11 coeluted in the chromatogram. Their sum can be almost completely attributed to the former isomer as CLA c9c11 is usually present in ruminant milk fat in very low amounts (Shingfield *et al*., 2010). When ALA-rich diets are fed to ruminants, CLA t11c13 (instead of t7c9) is usually reported as the second most abundant CLA isomer in milk from goats (Bernard *et al*., 2009b). CLA t11c13 and c9c11 were reported to significantly increase with linseed addition to goat's diets by many researchers (Bernard *et al*., 2009b; Chilliard and Ferlay, 2004; Marin *et al*., 2011), results which are confirmed in the study. Such response is consistent with

their biochemical way of synthesis. In fact, CLA t11c13 is thought to be formed at the third step of ruminal BH of ALA, by means of an isomerisation occurring at the expense of vaccelenic acid (Kraft *et al*., 2003). Moreover, >50% of CLA c9c11 was also reported to derive directly from ALA BH (Lee and Jenkins, 2011).

The unique way of synthesis of CLA t10c12 involves an isomerisation occurring at the first step of LA BH. This pathway, alternative to the more common forming RA, mainly occurs under dietary situations (e.g., high amounts of concentrate) that alter the rumen environment (Honkanen *et al*., 2012). In the current trial despite the low forage and high non-fibrous carbohydrates of the diets, CLA t10c12 was detected only in traces. This is in accordance with the results obtained by Bernard *et al*. (2009b) and Marin *et al*. (2011) and is consistent with the limited ruminal synthesis and mammary uptake of this FA in goats (Bernard *et al*., 2009a). Despite the higher estimated intake of LA in the goats fed the EL diet (Table 2), milk concentration of CLA t10c12 was not significantly different between groups. Such a result confirms findings of many previously published studies (Battacone *et al*., 2007; Bernard *et al*., 2009a, b; Li *et al*., 2012; Marin *et al*., 2011).

CLA t9t11 is exclusively formed within the rumen, probably mainly deriving from the LA BH and partly from ALA BH (Honkanen *et al*., 2012; Lee and Jenkins, 2011). As occurred in the study, Marin *et al*. (2011) also reported a lack of influence of dietary linseed on CLA t9t11 in goat milk. On the contrary, other studies showed that ALA-rich diets (e.g., pasture-based or linseed-supplemented) tend to increase the concentration of this isomer (Bernard *et al*., 2009a, b; Renna *et al*., 2012). Differences among studies may be the consequence of different total UFA intakes and different extents of ruminal BH with the experimental diets.

A large increase in goat milk CLA t10c12 and particularly t9t11 with dietary addition of linseed oil was found by Bernard *et al*. (2009b) which led them hypothesize an inhibitory role of these CLA isomers on Δ9-desaturase activity (as observed in dairy cows). The results obtained in the trial suggest that UFA and/or ruminal BH intermediate products other than CLA t10c12 and t9t11 may also exert inhibitory effects on mammary Δ9-desaturase activity in goats.

Effect of cheese-making and ripening on the fatty acid composition: Cheese-making and ripening usually have only minimal impact on the FA profile of dairy products, particularly if compared to the great influence determined by animal feeding (Chilliard and Ferlay, 2004). This is consistent with the lack of significant changes found in the study in almost all detected FA among milk, fresh and ripened cheeses. Other researchers who evaluated the effects of vegetable lipid supplemented diets and cheese manufacturing on dairy products from ruminants also obtained comparable results to those of the current study (Bodas *et al*., 2010; Dehareng *et al*., 2009). The observed significant reduction in CLA t9t11 between milk and ripened cheese may be ascribed to geometrical conversion between cis and trans double bonds and/or to positional isomerisation of CLA isomers which may occur during cheese processing (Buccioni *et al*., 2012).

The significant and positive linear correlations found among milk, fresh and ripened cheeses in the study also confirm previous findings (Lucas *et al*., 2006; Raynal-Ljutovac *et al*., 2008). These observations point out that the nutritional quality of goat cheeses essentially depend on the FA profile of the milk used for their manufacturing which in turn can be strongly modified by dietary manipulation (Ferlay *et al*., 2010).

CONCLUSION

The inclusion of 3 g unprotected EL per 100 g DM in diets for mid-lactating dairy goats fed a 40:60 F:C TMR containing rye silage as main forage component determined an increase of bulk milk fat content without altering bulk milk yield, protein and lactose contents. The oleaginous supplement noticeably influenced the FA composition of goat milk as well as that of fresh and ripened cheeses. The overall nutritional quality of lipids was enhanced by linseed supplementation due to significant reductions of hypercholesterolemic medium-chain SFA, significant reductions of the n6/n3 FA ratio, significant increases of some beneficial short-chain SFA and higher concentration of ALA. However, increasing dietary ALA markedly inhibited the Δ9-desaturase activity in the mammary gland which resulted in significant declines in the endogenous synthesis of Δ9-desaturase products including OA and RA, both of them being well known for their health benefit potential. Another negative effect of EL supplementation was the reduction of some BCFA, namely C15 iso and C16 iso which are potential anti-cancer agents. Extruded linseed supplementation does not seem a plausible strategy to improve the synthesis of very long-chain n3 FA because of the limiting availability of Δ6-desaturase enzyme necessary to convert ALA to stearidonic acid (C18:4 c6c9c12c15). Cheese-making and ripening only slightly modified the compositional variability of FA and consequently the nutritional quality, originally acquired in milk fat.

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