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Effect of dietary inclusion of lampante olive oil on milk and cheese fatty acid profiles of ewes

By **E. Vargas-Bello-Pérez**[1,*], **R. R. Vera**[1], **C. Aguilar**[1], **R. Lira**[1], **I. Peña**[1], **A. Valenzuela**[1] and **H. Cerda**[1]

[1] Departamento de Ciencias Animales, Pontificia Universidad Católica de Chile, Santiago, Chile Casilla-306. P. O. Box 6904411
* Corresponding author: evargasb@uc.cl

RESUMEN

Efecto de la inclusión de aceite de oliva lampante en la dieta de ovejas sobre el perfil de ácidos grasos de leche y queso

El objetivo de este estudio fue evaluar el efecto de la inclusión de aceite de oliva lampante en la dieta de ovejas sobre el perfil de ácidos grasos de leche y queso. Nueve ovejas fueron utilizadas en un diseño de CuadradoLatino 3 × 3. Las dietas fueron suplementadas con 0 (control; T0), 36 (T1) y 88 (T2) g de aceite de oliva lampante por kg de materia seca de alimento. La ingesta, la producción de leche y la composición de leche (grasa y proteína) no se vieron afectados por los tratamientos. Los ácidos oleico y vaccénico incrementaron ($P < 0.05$) gradualmente mientras que los ácidos grasos saturados y el índice aterogénico disminuyeron ($P < 0.05$) en leche y queso a medida que la inclusión de aceite de oliva lampante se incrementó en la dieta. En conclusión, la inclusión de aceite de oliva lampante en dietas de ovejas lactantes aumenta la concentración de ácidos grasos monoinsaturados y disminuye la de los saturados en leche y queso con posibles efectos benéficos sobre la salud humana.

PALABRAS CLAVE: Aceite de oliva – Ácidos grasos – Leche – Oveja – Queso.

SUMMARY

Effect of dietary inclusion of lampante olive oil on milk and cheese fatty acid profiles of ewes

The objective of this experiment was to evaluate the effect of a dietary supplementation of lampant olive oil on the fatty aid profiles of the milk and cheese of ewes. Nine lactating ewes were used in a 3 × 3 Latin square design. Dietary treatments were supplemented with 0 (control; T0), 36 (T1) and 88 (T2) g of lampante olive oil/kg of dry matter intake (DM). DM, milk yield and milk composition (fat and protein) were not affected by dietary treatments. Oleic and vaccenic acids gradually increased ($P < 0.05$) and the saturated FA and atherogenicity index decreased ($P < 0.05$) in milk and cheese as the concentration of lampante olive oil was increased in dietary rations. Overall, the supplementation of lampante olive oil in the diets of lactating ewes increased monounsaturated FA and decreased saturated FA concentrations in milk and cheese, thus improving their quality from the human health standpoint.

KEY-WORDS: Cheese – Ewe – Fatty acid – Milk – Olive oil.

1. INTRODUCTION

In the Mediterranean-type region of Chile, unirrigated areas are characterized by harsh conditions and around 77% of these lands are poor quality grasslands that are usually designated for sheep production. In these areas, dual purpose (*Merino Precoz*) and meat (*Suffolk and Hampshire*) breeds are of particular economic interest; however, there have been efforts to improve prolificity, carcass quality and feed conversion efficiency through the crossbreeding of *Merino Precoz* ewes with *Dorset* and *Sufflok* rams and crosses between *Merino Precoz* and *Finnish Landrace* (Crempien, 1999). Usually, in traditional sheep production systems of the dry areas of Chile, lambs are left with their mothers and they become valuable on the market since their diet is based solely on milk (Aguilar *et al*., 2006). These production systems can attain high gross profit margins but low profitability per hectare and farmers need to increase their profits by adding value to their products (Vera *et al*., 2009). The sheep growing region of Chile is interspersed with a rapidly increasing area sown with olive trees dedicated to high quality olive oil production, and the oil extracting plants produce large amount of various by-products, including considerable amounts of discarded oil (lampant olive oil).

The impact of supplementation with vegetable oils on sheep's milk fatty acid (FA) profile has been studied previously (Bessa *et al*., 2005; Bouattour *et al*., 2008; Gómez-Cortes *et al*., 2008a, 2009; Manso *et al*., 2009, 2011). Usually lipid-supplemented diets provoke decreases in dry matter intake mainly due to its palatability (Klevenhusen *et al*., 2011) and rumen effects. Gómez-Cortes *et al.* (2008b) reported that the supplementation of ewe diets with olive oil (60 g kg^{-1} of DM) does not have detrimental effects on animal performance but significantly modifies milk's FA profile. Also, Antongiovanni *et al*. (2002) and Martini *et al.* (2004) reported that feeding olive oil calcium soaps (50 g d^{-1} of DM and 7% as feed respectively) to dairy ewes can improve their milk FA characteristics (increased concentration of conjugated linoleic acid) towards a healthier pattern for human consumption.

Sheep's milk and cheese are gourmet products in Chile and there is an increasing demand from a growing and diversified market that includes an incipient sector of functional and nutraceutical foods. Despite the fact that there are previous reports on the use of olive cake silage (Sadeghi *et al*., 2009), olive oil (Gómez-Cortes *et al*., 2008b) and Ca salts of olive oil (Antongiovanni *et al*., 2002, Martini *et al*., 2004) in dairy ewe diets, there are no studies on the use of lampante olive oil in ewe (with dual purpose animals) diets under the conditions of the Mediterranean-type region of Chile. Therefore, the objective of this experiment was to evaluate the effect of the dietary supplementation of lampante olive oil on the fatty aid profiles of the milk and cheese of ewes.

2. MATERIALS AND METHODS

All animals were handled following the guidelines of the Animal Care Committee of the Pontificia Universidad Católica de Chile.

2.1. Animals and diets

Nine lactating ewes (crossbreed between Finnish Landrace, Border Leicester, Poll Dorset and Merino Precoz) (BW 56.5 ± 5.2 kg) with 45 d of lactation at the beginning of the experiment were assigned in a replicated (n = 3) 3 × 3 Latin Square design, that included three experimental periods of 10 d each (6 d of diet adaptation and 4 d of data collection) and three dietary treatments. The animals were housed in individual pens (2 m^2) with free access to water.

Their diets were elaborated according to the lactating ewe requirements of the National Research Council (2007). Three iso-caloric, iso-nitrogenous and iso-fibrous diets were formulated. The diets consisted of a total mixed ration (TMR), including molasses to avoid selection of dietary ingredients, based on alfalfa hay, ground corn grain and soybean expeller meal supplemented with 0 (control; T0), 36 (T1) and 88 g of lampante olive oil/kg of DM (T2). These amounts of lampante olive oil were chosen to mimic those supplied by olive oil cake in a parallel experiment (unpublished). Lampante olive oil was added daily to the TMR to avoid rancidity and allow for homogenous inclusion in the diet. The rations were supplied twice daily (1030 and 1830 h). Orts were measured daily during each collection period to determine feed intake for each ewe. Lampante olive oil was obtained from olive oil that is not suitable for human consumption due to its high acidity and defective aroma, color and taste (donated by Bethania, Chile) (EC regulation No. 2568, 1991).

2.2. Measurements, sample collection and chemical analyses

Ewes were milked manually at 0830 h every day. After milking, lambs were left with the ewes (to mimic real production conditions from the Mediterranean-type region of Chile) from 1300 to 1830 h and then housed in two 10 m^2 pens. During data collection periods, feed intake and milk yield were measured daily. Milk samples were obtained only from morning milking during the last 4 d of each experimental period, preserved with Bronopol™ (100mg $sample^{-1}$) and pooled for each collection period; approximately, 80 ml/ewe/period of milk sample were stored at –20°C for later analysis.

The diets were sampled during data collection periods and stored at –20°C for later chemical analyses. Standard procedures described by AOAC (2006) were used to determine the DM, Kjeldahl N, and ether extract. Crude protein was calculated as N × 6.25. Neutral detergent fiber and ADF were determined according to the methods described by Goering and Van Soest (1970) and Van Soest et al. (1991). Milk samples were analyzed for pH, titratable acidity (Thörner degrees; °Th), fat content (Gerber method; British Standards Institution 696, 1969), crude protein (Kjeldahl N × 6.38), and total ash and solids according to the AOAC (1984) procedures.

2.3. Fatty acid analysis

Lipids from the milk, cheese, lampante olive oil and diets were extracted with chloroform/methanol (2:1, v/v) according to the Folch *et al*., (1957) method and methylated with the modifications of Sukhija and Palmquist (1988). Analyses of the fatty acid methyl esters (FAME) of the experimental diets, olive oil and milk samples were performed using a gas chromatograph (GC; Shimadzu Scientific Instruments AOC-20s, Columbia, MD, USA) equipped with a 30-m fused-silica capillary column (Rtx 2330, 30m × 0.32mm i.d., 0.20 µm film thickness; Restek Corporation, Bellefonte, PA) but cheese fatty acids were determined using an Rtx column 100 m × 0.32 mm × 0.20 um column. The GC conditions were as follows: initial temperature of 40 °C, 2.5 °C min^{-1} to 226 °C, injector and detector temperatures were 260 °C, the column flow was 3.0 ml min^{-1}, the split ratio was 1:10 and 1 µL injection volume was used. The hydrogen carrier gas flow to the detector was 40.0 mL min^{-1}, the airflow was 400.0 mL min^{-1} and the flow of nitrogen makeup gas was 30.0 mL min^{-1}. Fatty acid (FA) peaks were identified by using two FAME standards (GLC 60; Nu-Check-Prep, Elysan, MN, USA), and the Food Industry 37 FAME mix, 35077 Restek Co, Bellefonte, PA, USA).

2.4. Cheese-making process

Milk from the same treatment and period across Latin squares was pooled and made into cheese. The cheeses were made in a pilot plant as follows: 5 L of milk were heated to 60 °C for 15 min, and commercial rennet was added to curdle the milk at 38 °C. No starter culture was added for cheese making. After the milk had clotted (30 min), the curd

was cut to the size of a hazelnut and then the vat temperature was gradually increased to 37 °C at a rate of 1°C 3 min^{-1} and maintained for 2 h.

At the same time, the curd was stirred to remove the whey and favor grain aggregation. The curds were placed into 350-g molds and pressed in a horizontal mechanic press until the pH was about 5.5. The cheese were salted in brine at 10 °C for 12 h and then transferred to a ripening room where they remained at a temperature of 9-10 °C and ~90% RH for 60 d. Three cheeses per treatment were allowed to mature for 60 d, at which time two cores from each cheese were removed for FA analysis.

2.5. Atherogenicity and thrombogenic indices

Atherogenicity (AI) and thrombogenic (TI) indices were calculated according to Ulbricht and Southgate (1991) formulas:

• AI = [(12:0 + 4(14:0) + 16:0] / [(n6 + n3)PUFA + + 18:1 + ΣMUFA]

• TI = (14:0 + 16:0 + 18:0) / [(0.5 × 18:1) + + 0.5(ΣMUFA) + 0.5(n6PUFA) + 3(n3PUFA) + + (n3PUFA/n6PUFA)].

Where MUFA = monounsaturated FA and PUFA = = polyunsaturated FA.

2.6. Statistical analyses

The data were analyzed as a replicated (n = 3) 3 × 3 Latin Square using the General Lineal Models procedure of SAS Statistical Package (SAS Institute Inc., 2004). The fixed effects were experimental periods and treatment; the random effect was the ewe. A probability of $P < 0.05$ was used to determine significant differences among means.

The statistical model was as follows:

$$Y_{ijkl} = \mu + \theta_i + D_{(i)j} + \rho_{(i)k} + \tau_l + \theta \cdot \tau i_l + E_{(ijkl)}$$

where Y_{ijkl} is the dependent variable, μ is the general mean, θ_i is the effect of the Latin square, $D_{(i)j}$ is the effect of animal within each Latin square, $\rho_{(i)k}$ is the effect of period in each Latin square, τ_l is the treatment effect, $\theta \cdot \tau_{il}$ is the corresponding interaction, and $E_{(ijkl)}$ is the experimental error.

In an effort to overcome the inevitable correlation among fatty acids, treatment profiles were initially analyzed with SAS multivariate analysis of variance, and since significant differences (not shown) were found among the respective vectors, univariate analyses were performed for individual fatty acids.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of ingredients and diets

The diets formulated for this experiment were iso-caloric (2.6 Mcal kg^{-1} ME), iso-nitrogenous (15% CP) and iso-fibrous (34% NDF) (Table 1).

Table 1
Ingredients and chemical composition of dietary treatments (DM basis)

Item	Dietary treatments[1]		
	T0	T1	T2
Ingredients (g kg^{-1})			
Alfalfa hay	669.4	663.1	683.7
Ground corn grain	237.3	215.3	179.8
Soybean expeller meal	46.6	58.7	48.8
Olive oil	0.0	15.3	38.7
Molasses	21.2	21.5	22.2
Sodium bicarbonate	21.2	21.5	22.2
Mineral mix[2]	4.2	4.3	4.4
Chemical composition (%)			
Dry matter	90.3	90.5	90.9
Ether extract	2.0	3.5	5.9
Crude protein	14.8	14.7	14.6
Neutral detergent fibre	34.0	35.4	34.3
Acid detergent fibre	22.2	22.8	21.5
Lignin	2.5	2.2	2.3
Ca[3]	1.3	1.3	1.2
P[3]	0.2	0.2	0.2
Ash	8.4	8.3	8.5
Digestible energy (Mcal kg^{-1})[4]	3.2	3.0	3.4
Metabolizable energy (Mcal kg^{-1})[5]	2.6	2.6	2.7

[1] Dietary treatments containing: T0 = 0, T1 = 36, T2 = 88 g/d of lampante olive oil; [2] Contained: Minerals (g kg^{-1}): Ca = 165; P = 83; Mg = 2; Mn = 5.2; NaCl = 246; Zn = 2; Micro minerals (mg/kg): Cu = 5.3; Co = 5.3; I = 20; S = 53; K = 6.5; Vitamins (UI kg^{-1}): A = 400,000; D_3 = 40,000; E = 22; [3] Analyzed by mass spectrometry (Liberty ICP instrument, Varian, Inc.); [4] Crampton *et al*., 1957; [5] Moe and Tyrrel, 1976.

3.2. Milk yield, milk composition and DMI

There was statistical evidence of treatment differences in dry matter intake (Table 2, P = 0.057) which tended to decrease with the highest inclusion of lampante olive oil (T2). Dietary supplementation with vegetable oils rich in unsaturated FA often results in a reduction in DMI (Shingfield *et al.,* 2006), which is related to potentially detrimental effects on ruminal fermentation such as reduced digestibility of non-lipid energy sources that is often accompanied by a reduced production of methane, hydrogen, and volatile FA (Jenkins, 1993).

In the Mediterranean-type region of Chile farmers often leave the ewes with their lambs after milking for several weeks to ensure adequate lamb growth while grazing low quality pastures. Milk consumed by suckling lambs was not estimated and that explains the low daily milk yield reported in this experiment.

Table 2
Milk yield, milk composition and dry matter intake from ewes supplemented with lampante olive oil

Item	Dietary treatments[1]			SD[2]	*P*-value[3]
	T0	T1	T2		
Dry matter intake (kg d^{-1})	2.37	2.37	2.26	0.05	0.057
Milk yield (mL d^{-1})	382.36	394.58	374.86	28.89	0.355
Milk composition (%)					
Fat	4.22	4.64	5.21	0.64	0.063
Crude protein	5.83	5.78	5.87	0.13	0.340
Total solids	16.08	16.13	16.57	0.81	0.473
Ash	0.97	0.97	0.99	0.03	0.111
Titratable acidity (°Th)	30.31	30.96	30.75	2.23	0.856
pH	6.34	6.41	6.32	0.09	0.213

[1] Dietary treatments containing: T0 = 0, T1 = 36, and T2 = 88 g/d of lampante olive oil; [2] Standard deviation of the grand mean; [3] *P*-value represents the probability of a treatment effect; [a,b,c] Means in the same row with different superscripts are different ($P < 0.05$).

Milk yield and milk composition were not affected by dietary treatments (36 and 88 g d^{-1} of lampante olive oil) which might be related to no negative effects of lampante olive oil on ruminal processes. This is in agreement with a Gómez-Cortés *et al.* (2008a) study in which no differences in the DMI, milk yield or milk composition in lactating ewes supplemented with soybean oil (60 g kg^{-1} DM) were reported. However, Gómez-Cortés *et al.* (2008b) observed that olive oil supplementation (60 g kg^{-1} DM) in dairy ewes did not affect DMI but increased milk yield. Hervás *et al.* (2008) reported no effect on ewes supplemented with sunflower oil (60 g kg^{-1} DM); however, they found an increase in milk fat content and decrease in milk protein content, which agrees in part with the tendency to increase milk fat content (Table 2; P = 0.063) as lampant olive oil was increased in the diet in this experiment.

Although it has been suggested that milk yield increases when sheep diets are supplemented with oils, these changes might be due to the greater energy contents of supplemented diets vs. non-supplemented standard diets (Chilliard *et al.*, 2003). The effects of oil supplementation on milk production, fat and protein yield depend on the genetic potential of the ewe to increase milk production as it consumes more dietary nutrients (Appeddu *et al.*, 2004). Manso *et al.* (2011) supplemented sheep diets with the same amount (3% DM) of hydrogenated palm, olive, soybean and linseed oils and fed animals with iso-nitrogenous and iso-energetic diets. They found no difference in milk yield or milk composition. Similarly, in the current experiment, animals were offered equal amounts of feed with the same ingredients. Hence, it is unlikely that milk composition would be affected by dietary energy and protein content differences, because all dietary treatments used were balanced to be iso-nitrogenous, iso-fibrous and iso-energetic.

3.3. Fatty acid composition of dietary treatments, milk and cheese

The fatty acid composition of lampante olive oil and dietary treatments are shown in Table 3. As expected, about 70% of the FA identified in the lampante olive oil used in this experiment was 18:1 *cis*-9.

Except for 4:0, 6:0, 8:0 and 17:0, the milk saturated FA (10:0 to 18:0) was affected by dietary treatments (Table 4). Compared with T0 and T1, T2 was higher ($P < 0.05$) in 18:1 *cis*-9, 18:1 *trans*-11, and 20:1 *cis*-11 and lower ($P < 0.05$) in 14:1 *cis*-9 and 17:1 *cis*-10. The supplementation of olive oil (TI and T2) reduced ($P < 0.05$) 18:2n6 *cis*-9, *cis*-12 and 18:3n3 cis-9, *cis*-12, *cis*-15 compared with T0. Myristic (14:0), palmitic (16:0) and oleic (18:1 *cis*-9) acids were the major FA present in milk samples. Oleic and stearic acids gradually rose ($P < 0.05$) in milk as the concentration of lampante olive oil increased in dietary rations. Following dietary supplementation with oils rich in long-chain FA , a reduction in short- and medium- chain FA contents in milk has been reported in studies on sheep (Zhang *et al.*, 2006; Chiofalo *et al.*, 2004; Gómez-Cortés *et al.*, 2008b) and goats (Schmidely and Sauvant, 2001). The decrease in medium-chain FA may represent an improvement in the profile of milk fatty acids because medium-chain FA have been reported to constitute the hypercholesterolemic portion of milk fat (Ney, 1991); however, a reduction in 6:0 to 10:0 may be undesirable because of their potential hypocholesterolemic effects. Unfortunately, the transfer of medium-chain FA from diet to milk is very inefficient; hence dietary supplementation is not useful for increasing these FA in milk (Grummer, 1991). Also, when the bioavailability of C18 FA increases as a result of increased dietary intake, 10:0 to 16:0 *de novo*

Table 3
Fatty acid composition of lampante olive oil and dietary treatments

Fatty acid (g 100 g^{-1})	Dietary treatments[1]			Lampante olive oil
	T0	T1	T2	
Saturated				
14:0	0.09	0.20	0.10	–
16:0	7.94	8.20	11.72	14.4
17:0	–	–	–	0.12
18:0	1.76	1.85	2.59	2.10
Monounsaturated				
16:1 *cis*-9	0.46	0.43	0.49	1.29
17:1 *cis*-10	–	–	–	0.26
18:1 *cis*-9	8.67	8.87	12.20	70.87
20:1 *cis*-11	–	0.01	0.01	0.33
Polyunsaturated				
18:2n6 *cis*-9, *cis*-12	24.70	24.94	32.99	7.3
18:3n6 *cis*-6, *cis*-9, *cis*-12	0.77	0.89	1.28	0.40
18:3n3 *cis*-9, *cis*-12, *cis*-15	11.94	11.57	16.52	0.69
20:3n6 *cis*-8, *cis*-11, *cis*-14	0.17	0.34	0.17	0.14

[1] Dietary treatments containing: T0 = 0, T1 = 36, and T2 = 88 g/d of lampante olive oil.

synthesis decreases as does their (10:0 to 16:0) concentration in milk (Gómez-Cortés *et al*., 2008b). Our findings agree with Chiofalo *et al.* (2004) who reported more 18:1 and 18:0 and less medium-chain saturated FA in milk of ewes supplemented with olive cake.

Compared with other studies (Antongiovanni *et al*., 2002; Martini *et al*., 2004; Gómez-Cortes *et al*., 2008b; Sadeghi *et al*., 2009), the concentrations of short-chain FA found in milk appeared to be low, however, the nature (FA composition), the type (oilseeds, protected or unprotected oils) and the amount of dietary fat, and their interactions with the basal diet ingredients have been found to affect the ruminal biohydrogenation process (Chilliard *et al*., 2000).

In the current experiment, the contents of 10:0-17:0, 18:2n6 *cis*-9, *cis*-12 and 18:3n3 *cis*-9, *cis*-12, *cis*-15 were diminished and 18:0, 18:1 *trans*–11 and 18:1 *cis*-9 were increased in cheese as lampante olive oil supplementation increased (Table 5). The higher levels of 18:1 *cis*-9 observed in the milk and cheese of ewes fed T2 diet compared with those in ewes fed T0 and T1, may be partly due to the action of the Δ^9-desaturase enzyme in the mammary gland (Griinari *et al*., 2000) over a portion of 18:0 produced by ruminal biohydrogenation, and partly to the fact that T2 contains higher concentrations of oleic acid than T0 and T1. In ruminants, roughly 40% of 18:0 absorbed by the mammary gland is desaturated to 18:1 *cis*-9 (Lock and Garnsworthy, 2003). The increase in monounsaturated FA milk content found in this experiment also agrees with other studies which used unprotected olive oil (Gómez-Cortés *et al*., 2008b) and olive oil calcium soap (Antongiovanni *et al*., 2002; Martini *et al*., 2004).

Compared with T0; SFA, PUFA, PUFA n-3, and PUFA n-6 in the milk were lower ($P < 0.05$) in T1 and T2. Monounsaturated (MUFA), and MUFA/SFA and PUFA n-6/PUFA n-3 ratios were higher ($P < 0.05$) in T2 than in T0 and T1 (Table 6). The reduction in PUFA content in the milk from ewes fed T1 and T2 was due to reduced contents of 18:2n6 *cis*-9, *cis*-12 and 18:3n3 *cis*-9, *cis*-12, *cis*-15. Conversely, Antongiovanni *et al*. (2002), reported no differences in 18:2n6 *cis*-9, *cis*-12 and 18:3n3 *cis*-9, *cis*-12, *cis*-15 contents in the milk from ewes fed olive oil calcium soap. Martini *et al*. (2004) reported a decrease in 18:2n6 *cis*-9, *cis*-12 and an increase in 18:3n3 *cis*-9, *cis*-12, *cis*-15 contents in milk from ewes fed olive oil calcium soap. On the other hand, Gómez-Cortés *et al.* (2008b) reported a decrease in both FA; they attributed those results to the reduced dietary contents of those FA.

The MUFA/SFA ratio in milk and cheese was higher in T2 than in T0 and T1, which may be explained by a combined effect between the increases in unsaturated FA (mainly MUFA) and decreases in SFA. Similar results were reported by Antongiovanni *et al.* (2002), Martini *et al.* (2004), and Hervás *et al.* (2008). Despite the fact that PUFA n-6 and PUFA n-3 were reduced (12.3 and 47.5% respectively) from T0 to T2, the PUFA n-6/PUFA n-3 ratio in milk was increased in T2. One of the main considerations when trying to meet

Table 4
Effect of treatment diets on the fatty acid composition of the milk of ewes supplemented with lampante olive oil

Fatty acid (g 100 g^{-1})	Dietary treatments[1]			SD[2]	*P*-value[3]
	T0	T1	T2		
Saturated					
4:0	1.14	1.21	1.19	0.08	0.608
6:0	1.39	1.42	1.33	0.10	0.168
8:0	1.74	1.77	1.60	0.17	0.117
10:0	7.93[a]	7.51[b]	6.47[b]	0.79	0.009
11:0	0.28[a]	0.26[a]	0.17[b]	0.08	0.042
12:0	6.14[a]	5.29[b]	4.33[c]	0.61	0.004
14:0	14.66[a]	13.56[b]	12.23[c]	0.82	0.007
15:0	1.66[a]	1.38[b]	1.30[b]	0.12	0.001
16:0	33.85[a]	31.79[b]	30.62[b]	1.79	0.026
17:0	0.70	0.60	0.58	0.09	0.111
18:0	6.12[c]	8.31[b]	9.67[a]	1.11	0.001
Monounsaturated					
14:1 *cis*-9	0.85[a]	0.72[b]	0.65[c]	0.05	< 0.001
16:1 *cis*-9	1.55	1.42	1.41	0.10	0.032
17:1 *cis*-10	0.33[a]	0.18[b]	0.15[c]	0.09	0.020
18:1 *cis*-9	15.91[c]	18.47[b]	21.54[a]	1.51	0.002
18:1 *trans*-11	1.53[c]	2.36[b]	2.99[a]	0.65	0.014
20:1 *cis*-11	0.75[b]	0.93[b]	1.33[a]	0.28	0.012
Polyunsaturated					
18:2n6 *trans*-9, *trans*-12	0.37	0.38	0.42	0.09	0.550
18:2n6 *cis*-9, *cis*-12	2.08[a]	1.79[b]	1.46[c]	0.12	< 0.001
18:3n6 *cis*-6, *cis*-9, *cis*-12	0.18	0.20	0.16	0.12	0.780
18:3n3 *cis*-9, *cis*-12, *cis*-15	0.51[a]	0.41[b]	0.29[b]	0.11	0.018

[1] Dietary treatments containing: T0 = 0, T1 = 36, and T2 = 88 g/d of lampante olive oil; [2] Standard deviation of the grand mean; [3] *P*-value represents the probability of a treatment effect; [a,b,c] Means in the same row with different superscripts are different ($P < 0.05$).

current recommendations for PUFA n-3 is that the current intake of PUFA consists primarily of PUFA n-6. The competition for desaturases and elongases in PUFA n-6 and PUFA n-3 metabolism results in inverse affects on tissue concentrations of these FA (Gebauer *et al*., 2006). The results obtained from PUFA n-6 and PUFA n-3 content in milk and cheese from ewes fed T2, are important because they show that milk from ewes fed with lampante olive oil could be used to produce cheese with enhanced FA quality (Manso *et al*., 2011).

The atherogenicity index in milk was reduced ($P < 0.05$) as dietary lampante olive oil supplementation increased (Table 6). The atherogenicity index was lower in milk and cheese obtained from ewes fed with the highest level of lampante olive oil inclusion in the diet (T2). Similarly, Castro *et al*. (2009) reported a reduction in this index when lactating ewes were supplemented with 12 g kg^{-1} of sunflower oil. In the human diet, lipids (particularly SFA) are known to contribute to coronary heart disease (CHD; Williams, 2000). Conversely, some milk unsaturated FA can diminish the risk of cardiovascular disease, including 18:2 *cis*-9, *trans*–11, MUFA (in particular 18:1 *cis*-9), and PUFA. In the present experiment, the addition of lampante olive oil in lactating ewes' diets decreased most SFA. The recognized atherogenic SFA are 12:0, 14:0, and 16:0 (Keys *et al*., 1965) with 14:0 being the most atherogenic, with about four times more cholesterol-raising potential than 16:0 (Ulbricht and Southgate, 1991). The present experiment showed that atherogenic FA in milk were reduced with the inclusion of lampante olive

Table 5
Effect of treatment diets on the fatty acid composition of the cheese from ewes' milk supplemented with lampante olive oil

Fatty acid (g/100 g)	Dietary treatments[1]			SD[2]	*P*-value[3]
	T0	T1	T2		
Saturated					
4:0	0.93	0.90	0.96	0.05	0.190
6:0	1.16	1.17	1.18	0.07	0.950
8:0	1.48	1.54	1.46	0.11	0.410
10:0	7.08^{a}	6.97^{a}	6.14^{b}	0.40	0.003
11:0	0.17^{a}	0.14^{ab}	0.11^{b}	0.02	0.016
12:0	5.72^{a}	5.17^{b}	4.26^{c}	0.27	< 0.001
13:0	0.17^{a}	0.14^{ab}	0.12^{b}	0.01	0.003
14:0	14.78^{a}	13.77^{b}	12.21^{c}	0.55	< 0.001
15:0	1.66^{a}	1.39^{b}	1.26^{b}	0.10	< 0.001
16:0	35.81^{a}	32.79^{b}	30.90^{b}	1.41	< 0.001
17:0	0.72^{a}	0.61^{b}	0.56^{b}	0.03	< 0.001
18:0	6.58^{b}	8.44^{a}	9.37^{a}	0.71	< 0.001
21:0	0.14	0.13	0.12	0.01	0.076
23:0	0.11^{a}	0.86^{a}	0.04^{b}	0.07	0.005
Monounsaturated					
14:1 *cis*-9	0.82^{a}	0.70^{b}	0.63^{b}	0.07	0.001
15:1 *cis*-10	0.26^{a}	0.24^{b}	0.19^{b}	0.22	0.022
16:1 *cis*-9	1.52	1.46	1.39	0.17	0.490
17:1 n3	0.32^{a}	0.27^{b}	0.24^{b}	0.03	0.003
18:1 *cis*-9	15.00^{c}	18.01^{b}	21.53^{a}	1.44	< 0.001
18:1 *trans*-11	1.47^{c}	2.14^{b}	3.08^{a}	0.59	0.001
20:1 *cis*-9	0.68^{b}	0.95^{b}	1.40^{a}	0.19	< 0.001
Polyunsaturated					
18:2n6 *trans*-9, *trans*-12	0.33^{b}	0.38^{b}	0.44^{a}	0.03	< 0.001
18:2n6 *cis*-9, *cis*-12	1.97^{a}	1.72^{b}	1.48^{c}	0.12	< 0.001
18:3n6 *cis*-6, *cis*-9, *cis*-12	0.28	0.29	0.29	0.01	0.721
18:3n3 *cis*-9, *cis*-12, *cis*-15	0.43^{a}	0.36^{b}	0.31^{b}	0.03	< 0.001
20:3n6	0.17^{a}	0.15^{b}	0.13^{b}	0.01	< 0.001
20:3n3	0.18^{a}	0.15^{b}	0.13^{b}	0.01	0.008

[1]Dietary treatments containing: T0= 0, T1= 36, and T2= 88 g/d of lampante olive oil; [2]Standard deviation of the grand mean; [3]Represents the probability of a treatment effect; [a,b,c] Means in the same row with different superscripts are different ($P < 0.05$).

oil in ewe diets suggesting a positive health effect for human consumption. Despite the fact that 14:0 and 16:0 and PUFA were diminished and 18:0 and 18:1 *cis*-9 were increased in the milk from ewes fed T2, the thrombogenic index was not different between dietary treatments suggesting that there was a biological distinction among different FA that are accounted for in this index.

4. CONCLUSIONS

Overall, the supplementation of lampante olive oil (36 or 88 g d^{-1}) to lactating ewes' diets can increase the contents of 18:1 *cis*-9 and 18:1 *trans*–11, and reduce the atherogenicity index in their milk and cheese . The results from the present experiment support the possibility of implementing

Table 6
Effect of treatment diets on the major fatty acid (FA) classes in the milk and cheese of ewes supplemented with lampante olive oil

Fatty acid (g/100 g)	Dietary treatments[1]			SD[2]	*P*-value[3]
	T0	T1	T2		
Milk					
Saturated (SFA)	75.79[a]	73.13[b]	69.65[b]	2.49	0.012
Monounsaturated (MUFA)	20.93[b]	24.08[b]	28.07[a]	2.24	0.004
Polyunsaturated (PUFA)	3.28[a]	2.79[a]	2.38[b]	0.42	0.017
PUFA n-3	0.59[a]	0.43[b]	0.31[b]	0.15	0.046
PUFA n-6	2.69[a]	2.37[b]	2.36[b]	0.28	0.011
MUFA/SFA	0.32[b]	0.37[b]	0.44[a]	0.04	0.012
PUFA n-6/PUFA n-3	4.76[b]	5.78[b]	7.67[a]	1.62	0.010
Atherogenicity index[4]	4.13[a]	3.43[b]	2.78[c]	0.47	< 0.001
Thrombogenic index[5]	1.59	1.69	1.78	0.30	0.450
Cheese					
Saturated (SFA)	76.62[a]	73.26[b]	68.65[c]	1.80	< 0.001
Monounsaturated (MUFA)	20.02[c]	23.71[b]	38.53[a]	1.79	< 0.001
Polyunsaturated (PUFA)	3.37[a]	3.04[b]	2.81[b]	0.16	< 0.001
PUFA n-3	0.94[a]	0.77[b]	0.71[b]	0.07	< 0.001
PUFA n-6	2.75[a]	2.53[ab]	2.35[b]	0.15	0.001
MUFA/SFA	26.22[c]	32.40[b]	41.62[a]	3.23	< 0.001
PUFA n-6/PUFA n-3	2.92[b]	3.28[a]	3.34[a]	0.10	0.037
Atherogenicity index[4]	4.00[a]	0.93[b]	0.84[c]	0.02	< 0.001
Thrombogenic index[5]	1.62	1.59	1.45	0.15	0.145

[1] Dietary treatments containing: T0 = 0, T1 = 36, and T2 = 88 g/d of lampante olive oil; [2] Standard deviation of the grand mean; [3] *P*-value represents the probability of a treatment effect; [4] Atherogenicity index = [(12:0 + 4(14:0) + 16:0] / [(n6+n3)PUFA + 18:1 + ΣMUFA] (Ulbricht and Southgate, 1991); [5] Thrombogenic index = (14:0 + 16:0 + 18:0) / [(0.5 × 18:1) + 0.5(ΣMUFA) + 0.5(n6PUFA) + 3(n3PUFA) + + (n3PUFA/n6PUFA)] (Ulbricht and Southgate, 1991); [a,b,c] Means in the same row with different superscripts are different ($P < 0.05$).

a management system to produce sheep milk with a naturally enhanced FA composition that has a positive impact on cheese fatty acid quality.

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