JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

DOI:10.1021/jf101760u

Comparison of the Fatty Acid Profiles in Cheeses from Ewes Fed Diets Supplemented with Different Plant Oils

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The purpose of this work was to obtain a cheese from ewes milk with a healthier fatty acid (FA) profile. To achieve our aim, 48 ewes (12 per treatment) were fed diets supplemented with 3% of plant oils: palm (used as control), olive (OO), soybean (SO), and linseed (LO). Milk samples from each treatment were collected to manufacture cheeses. The cheesemaking process did not modify the dairy fat FA profile, but OO, SO, and LO did reduce the C12:0 + C14:0 + C16:0 content in dairy fat, thus decreasing the atherogenic index value in the cheeses. Percentages of *cis*-9 *trans*-11 C18:2 in cheeses ranged from the 0.43 control value to 0.92, 1.64, and 2.71 with OO, LO, and SO respectively, following the same pattern as *trans*-11 C18:1. In contrast, *trans*-10 C18:1 levels were always below 1%. The lowest n-6/n-3 ratio obtained with LO (1.43) suggests that such lipid supplementation would be the most effective nutritional strategy for improving cheese FA profiles.

KEYWORDS: Fatty acid; plant oil; milk fat; cheese; ewe

INTRODUCTION

Lipid composition is a paramount factor in the nutritional quality of cheeses. Alimentary guidelines have usually recommended that the amounts of saturated fatty acids (FAs) in dairy fat, especially C12:0, C14:0, and C16:0, should be reduced because of their potential hypercholesterolemic effects and that this should be done concurrently with an increase in polyunsaturated FAs. A considerable number of experiments over the past decade have analyzed the effect of ruminant diet supplementation with different fat sources on milk FA profiles (*1*−*3*). Plant oils from diverse oilseeds have a characteristic FA composition and accordingly have different influences on milk FA profiles. Even though extensive information on this matter is available, it is often difficult to choose the appropriate supplement to improve the nutritional characteristics of dairy fat, mainly because experimental conditions vary so greatly (i.e., the use of unequal basal diets, the physical form of the supplements, or the amounts added to the rations).

The incorporation of processed oilseeds (extruded, rolled, micronized, roasted, etc.) in ruminant diets has proved to be more effective than the use of intact seeds to increase milk conjugated linoleic acid (CLA), but less efficacious than free oil (*4*, *5*). However, adding high doses of oil to bovine rations is frequently discouraged because it could alter ruminal lipid metabolism and negatively affect milk production and FA composition (*6*).

Although milk fat from dairy ewes is of major economic relevance in the Mediterranean and Middle East countries, as it is used essentially for cheesemaking (*7*), agreements about the most convenient diet for increasing potentially healthy FAs in ewes milk fat is still an unresolved challenge. The purpose of this study was to evaluate the effect that adding 3% in dry matter (DM) of different plant oils to the diet under similar experimental conditions would have on the FA profile of ewes milk. Our aim has been to focus on ways of increasing vaccenic acid (VA, *trans*-11 C18:1), rumenic acid (RA, *cis*-9 *trans*-11 C18:2), and n-3 FAs without increasing saturated FAs, and especially *trans*-10 C18:1, a FA that could be linked to cardiovascular disease risk and which can be found in cheeses made from ewe milk.

MATERIALS AND METHODS

Animals and Dietary Treatments. Forty-eight pregnant Churra ewes (mean body weight, BW, 64.3 ± 0.92 kg) were selected before lambing and were fed on the same diet. The ewes aged 3−5 years, whose parity ranged from 4 to 6, all gave birth 3−4 days before starting the experiment. After lambing, each ewe was assigned to one of four treatments (12 ewes per treatment) based on their milk production, age, initial BW, and parity in randomization. All animal handling practices followed the recommendations of the European Council Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Valladolid (Spain). Each ewe received 32.6 g of a total mixed ration (TMR) diet per kg BW (on average 2.1 kg/ewe) comprised of lucerne (43%), maize (16%), barley (13%), soybean meal (13%), sugar beet pulp (10%), molasses (4%), and a vitamin mineral premix (1%). The chemical composition of the TMR was determined by using the procedures described by the AOAC (*8*) and was as follows: 86.9% DM, ash 6.95% DM, neutral detergent fiber 33.4% DM, acid detergent fiber 21.8% DM, crude protein 14.9% DM, and ether extract 1.75% DM. Moreover, each ewe received 3% of the corresponding oil added daily to the TMR: hydrogenated palm oil (control), olive oil (OO), soybean oil (SO), or linseed oil (LO). TMR was supplied twice a day together with 8% of barley straw, and fresh water was always available. The FA composition of the oils is shown in **Table 1**.

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Published on Web 09/13/2010

Milk Sampling and Cheesemaking. The animals were milked twice daily (at 0800 and 1900 h) in a 2 × 24 low-line Casse system milking parlour with 12 milking units and two milkers. The milking machine (Alfa-Laval Iberia, SA, Madrid, Spain) was set to provide 180 pulsations per minute in a 50:50 ratio with a vacuum level of 36 kPa. On days 48 and 54 of lactation, individual ewe milk production was recorded and samples were taken in milk collection jars. One subsample of milk was kept at 4 °C until analyzed for fat, protein, and lactose content in accordance with International Dairy Federation (*9*) standards, using a MilkoScan analyzer (FOSS Electric A/S, Hillerød, Denmark). Another subsample was stored at −80 °C for FA analysis. On day 55 of lactation, three cheeses were elaborated with milk from each treatment (one cheese for every four ewes), and a milk sample was also taken for chemical and FA analyses.

For cheesemaking, raw milk was heated to 32 °C, inoculated with a natural microbial culture (Choozit MA, Laboratorios Arroyo SA, Santander, Spain), which was composed of strains of *Lactococcus lactis* (ssp. *lactis*, *cremoris* and *lactis* var. *diacitylacis*) and *Streptococcus salivarius* ssp. *thermophilus*, and allowed to coagulate for 1 h with calf rennet paste (0.3 mL/l milk, Laboratorios Arroyo SA, Santander, Spain). The resultant curd was cut to obtain lumps of 5−10 mm, which were placed in cylindrical molds, pressed 1.5 kg for 1 h, 2.5 kg for 1 h, 3 kg for half an hour, salted in brine for 6 h, and matured for 60 days. The average pH of cheeses was 5.3 on the day of production ($P > 0.10$). Cheeses contained 35% fat, 26% protein, and 69% total solids and a pH of 5.1 after 60 days of aging, without statistical differences between experimental treatments ($P > 0.10$).

Fatty Acid Analysis. Milk fat separation was carried out using the method proposed by Luna et al. (*10*). Thirty mL of raw milk were centrifuged at 12000 rpm for 30 min at 20 °C in a Beckman (Fullerton, CA) J2-MC centrifuge. The fat layer was transferred to a microtube and then microcentrifuged at 13000 rpm for 20 min at room temperature. After this centrifugation, the top layer was taken to be analyzed. Cheese fat extraction was carried out according to ISO-IDF (*11*) using *n*-pentane into the chamber of the Soxhlet apparatus after grind the sample with a mixture of sand and sodium sulfate.

Table 1. Main Fatty Acid Composition of the Experimental Oils

	oil			
fatty acid	palm	olive	soybean	linseed
12:0	0.12	<0.10	0.10	<0.10
14:0	1.30	<0.10	0.20	0.10
15:0	0.10	<0.10	<0.10	<0.10
16:0	66.20	10.60	11.30	6.20
16:1	<0.10	0.80	0.20	0.10
18:0	31.00	4.00	4.00	4.90
18:1	<0.10	76.80	24.10	21.90
18:2	0.10	6.00	52.40	14.80
18:3	<0.10	0.70	6.20	51.30
20:0	0.50	0.40	0.40	0.20
22:0	0.10	0.10	0.50	0.10

Separated lipids were stored in amber vials, blanketed with a stream of N_2, and stored at −20 °C until analysis. Fatty acid methyl esters (FAME) were prepared by base-catalyzed methanolysis of the glycerides with KOH in methanol (*12*). Analysis of FAME was performed on a gas−liquid chromatograph (Agilent 6890 N Network System, Palo Alto, CA) by split injection (1:100) onto a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm, Varian, Middelburg, Netherlands). A gradient temperature program was used. The initial oven temperature was 160 °C. After 80 min, it was raised 10 °C/min to 210 °C and then held for 35 min. Helium was the carrier gas, and the injector and detector were at 250 °C. FAME correction factors were determined by analyzing butter oil of a certified FA profile (CRM 164; European Community Bureau of Reference, Brussels) as ISO-IDF (*13*) described. FA identification was made by comparison with analogous dairy fat samples and standard mixtures purchased from Nu-Chek Prep. Inc. (Elysian, MN).

Statistical Analysis. Data regarding individual milk production along with chemical and FA composition were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model took into account the fixed effects of dietary treatment, time, and their interaction. Time was considered a repeated factor and animal nested to treatment was subjected to a compound symmetry-covariance structure. Milk and cheese FA profiles were compared using a two-way ANOVA test with dietary treatment and milk or cheese as sources of variation. As the interaction was no significant in most cases, only means for the principal effects are presented in the tables.

RESULTS AND DISCUSSION

Animal Performance. The average daily milk yield and composition are recorded in **Table 2**. As can be seen, despite the type of oil added to the diet, animal performance was not negatively affected. Indeed, no changes were observed in milk yield or in composition, but there was an increase in milk fat percentage ($P < 0.01$) for OO ewes. Previous works showed a tendency of OO to increase milk and total solid yield although without affecting fat percentage (*14*).

Milk Fatty Acid Profile. Table 3 shows the FA profile of milk fat from ewes fed the different experimental diets. Milk FA composition was substantially altered by the type of vegetable oil administered. According to the FA composition of the oils (**Table 1**), control milk contained the highest levels of saturated FAs. The most abundant saturated FA in the control milk was palmitic acid, which reached values of up to 29% of total FAME (**Table 3**). In contrast to the control, the addition of oils rich in unsaturated FAs reduced significantly short- and medium-chain saturated FAs in ewe milk fat (**Table 3**). This pattern has already been reported in ewes milk fed diets enriched in oleic (*14*), linoleic (*15*, *16*), and α-linolenic (*17*−*19*) acids. Fatty acids from C6:0 to C14:0 and part of C16:0 are synthesized de novo in the mammary gland, and their decrease could be attributed to a

Table 2. Milk Production and Chemical Composition of Milk from Ewes Fed with a Supplement (3% in DM) of Palm (Control), Olive (OO), Soybean (SO), and Linseed (LO) Oils[a]

	treatment					*P* value		
	control	OO	SO	LO	SED	treatment	day	treatment × day
				Yield (g/d)				
milk	1242	1288	1321	974	161.7	ns	*	ns
fat	103.8	120.3	111.9	96.5	15.20	ns	ns	ns
protein	64.0	67.9	68.2	55.2	8.17	ns	ns	ns
lactose	57.3	59.2	59.8	49.0	8.16	ns	ns	ns
				Composition (%)				
fat	8.43 b	9.55 a	8.37 b	8.77 b	0.374	**	ns	ns
protein	5.18	5.35	5.23	5.29	0.121	ns	ns	ns
lactose	4.61	4.56	4.48	4.44	0.117	ns	ns	ns

[a] SED = standard error of difference. *P* value: *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; ns = $P > 0.05$. a,b: Different letters indicate significant differences ($P < 0.05$).

dilution effect generated by the uptake of FAs with 18-C atoms in the udder. Furthermore, the presence of some of these long chain FAs in the mammary gland could inhibit the activity of certain enzymes involved in de novo synthesis (*20*).

The greater concentration of C18:0 observed in OO, SO, and LO diets, compared with control, could also be attributed to the intake of unsaturated FAs ($P < 0.001$; **Table 3**). The predominant FAs in the different supplements (oleic, linoleic, and

Table 3. Fatty Acid Profile (g/100 g of Total Fatty Acid Methyl Esters) of Milk Fat from Ewes Fed with Supplements (3% of DM) of Palm (Control), Olive (OO), Soybean (SO), and Linseed (LO) Oils[a]

	treatment					*P* value		
	control	OO	SO	LO	SED	treatment	day	treatment × day
				SFA				
4:0	3.91	4.05	4.10	4.00	0.155	ns	ns	ns
6:0	3.37 a	2.88 b	2.75 b	2.97 b	0.178	***	ns	ns
8:0	3.14 a	2.47 b	2.29 b	2.58 b	0.249	***	ns	ns
10:0	9.41 a	6.68 b	6.03 b	6.90 b	0.524	***	ns	ns
12:0	5.05 a	3.57 b	3.30 b	3.65 b	0.374	***	ns	ns
13:0 *iso*	0.03 a	0.02 b	0.02 b	0.02 b	0.003	**	ns	ns
13:0 *anteiso*	0.05 a	0.03 b	0.03 b	0.03 b	0.004	***	ns	ns
13:0	0.18 a	0.12 b	0.11 b	0.11 b	0.015	***	**	ns
14:0 *iso*	0.11 a	0.07 b	0.08 b	0.08 b	0.009	***	ns	ns
14:0	10.93 a	9.60 b	9.02 c	9.74 b	0.413	***	ns	ns
15:0 *iso*	0.28 a	0.21 b	0.21 b	0.22 b	0.011	***	ns	ns
15:0 *anteiso*	0.46 a	0.31 b	0.34 c	0.32 b	0.021	***	ns	ns
15:0	1.01 a	0.70 b	0.73 b	0.72 b	0.033	***	ns	ns
16:0 *iso*	0.27 a	0.18 b	0.20 b	0.19 b	0.021	***	ns	ns
16:0	29.05 a	22.70 b	22.33 bc	21.46 c	0.771	***	ns	ns
17:0	0.53 a	0.41 c	0.44 b	0.43 bc	0.019	***	*	ns
18:0 *iso*	0.06 a	0.04 b	0.06 a	0.04 b	0.005	***	*	ns
18:0	7.99 c	11.55 a	10.19 b	11.40 a	0.833	***	ns	ns
19:0	0.08 a	0.05 b	0.04 c	0.04 c	0.006	***	**	ns
20:0	0.22 a	0.23 a	0.22 a	0.19 b	0.012	***	*	ns
21:0	0.07 a	0.05 b	0.05 b	0.04 c	0.004	***	ns	ns
22:0	0.11 b	0.09 c	0.13 a	0.08 c	0.006	***	ns	ns
23:0	0.08 a	0.05 b	0.06 b	0.05 b	0.005	***	ns	ns
24:0	0.05 a	0.03 c	0.04 b	0.03 c	0.003	***	*	ns
				MUFA				
10:1	0.38 a	0.25 b	0.23 b	0.25 b	0.023	***	ns	ns
cis-9 14:1	0.16	0.15	0.15	0.13	0.019	ns	ns	ns
15:1	0.12 a	0.09 b	0.09 b	0.09 b	0.006	***	ns	ns
trans-9 16:1 + 17:0 *iso*	0.38 c	0.40 c	0.71 a	0.55 b	0.048	***	ns	ns
cis-7 16:1	0.24 c	0.32 a	0.24 bc	0.26 b	0.013	***	**	ns
cis-9 16:1 + 17:0 *anteiso*	1.25 a	0.97 b	0.95 b	0.83 c	0.064	***	ns	ns
cis-13 16:1	0.10 a	0.05 b	0.05 b	0.06 b	0.011	***	ns	ns
17:1	0.18 a	0.14 b	0.13 b	0.12 c	0.010	***	ns	ns
trans (6 + 7 + 8) 18:1	0.26 c	1.01 a	0.49 b	0.43 b	0.053	***	ns	ns
trans-9 18:1	0.21 d	0.76 a	0.44 b	0.38 c	0.041	***	ns	ns
trans-10 18:1	0.27 c	0.79 a	0.77 a	0.45 b	0.087	***	ns	ns
trans-11 18:1 (VA)	0.78 d	2.08 c	6.52 a	4.27 b	0.626	***	ns	ns
trans-12 18:1	0.27 c	0.81 a	0.61 b	0.57 b	0.040	***	***	ns
cis-9 18:1	14.18 c	21.11 a	16.05 b	16.24 b	0.865	***	ns	ns
cis-11 + *trans*-15 18:1	0.38 c	0.59 b	0.66 a	0.66 a	0.029	***	***	ns
cis-12 18:1	0.18 c	0.16 c	0.83 a	0.53 b	0.076	***	ns	ns
cis-13 18:1	0.04 b	0.05 b	0.07 a	0.07 a	0.005	***	ns	ns
cis-14 + *trans*-16 18:1	0.27 d	0.36 c	0.47 b	0.62 a	0.030	***	ns	ns
cis-15 18:1	0.06 c	0.08 c	0.13 b	0.32 a	0.025	***	ns	ns
cis-16 18:1	0.03 b	0.03 b	0.03 b	0.05 a	0.003	***	*	*
cis-9 20:1	0.02 c	0.05 a	0.05 a	0.03b	0.003	***	*	ns
24:1	0.01	0.01	0.01	0.01	0.002	ns	***	ns
				Nonconjugated 18:2				
trans-11 *trans*-15	0.02 c	0.02 c	0.05 b	0.22 a	0.021	***	**	*
trans-9 *trans*-12	0.04 d	0.06 c	0.08 b	0.11 a	0.009	***	ns	ns
trans-8 *cis* 12 + cis-9 *trans*-13	0.09 d	0.12 c	0.16 b	0.23 a	0.017	***	ns	*
trans-8 *cis*-13	0.07 c	0.08 bc	0.09 b	0.16 a	0.010	***	ns	ns
cis-9 *trans*-12	0.03 b	0.04 a	0.03 b	0.05 a	0.006	***	ns	ns
trans-9 *cis*-12	0.03 c	0.03 c	0.05 b	0.08 a	0.005	***	***	***
trans-11 *cis*-15	0.07 c	0.06 c	0.22 b	1.36 a	0.105	***	***	***
cis-9 *cis*-12	1.99 b	1.51 c	3.17 a	1.76 bc	0.181	***	ns	ns
other 18:2	0.05 b	0.03 c	0.03 c	0.08 a	0.006	***	***	***

Table 3. Continued

	treatment					P value		
	control	OO	SO	LO	SED	treatment	day	treatment × day
				Conjugated 18:2				
cis-9 *trans*-11 (RA)	0.39 d	0.91 c	2.58 a	1.59 b	0.231	***	ns	ns
trans-9 *cis*-11	0.01 b	0.01 a	0.02 a	0.01 b	0.002	***	ns	ns
trans-10 *cis*-12	0.00 b	0.00 b	0.01 a	0.01 a	0.002	***	***	ns
trans-11 *cis*-13	0.01 b	0.01	0.03 b	0.19 a	0.034	***	ns	ns
trans-12 *trans*-14	0.01 b	0.01 b	0.02 b	0.07 a	0.006	***	ns	ns
trans-11 *trans*-13	0.03 b	0.02 d	0.02 c	0.08 a	0.004	***	**	ns
other *trans*−*trans*	0.01 c	0.01 c	0.03 a	0.02 b	0.003	***	ns	ns
				Other PUFA				
18:3 n-6	0.04 a	0.02 c	0.03 b	0.02 c	0.004	***	ns	ns
18:3 n-3	0.52 b	0.36 c	0.53 b	1.07 a	0.066	***	*	ns
cis-9 *trans*-11 *cis*-15 18:3	0.03 b	0.03 b	0.05 b	0.21 a	0.022	***	*	*
cis-9 *trans*-11 *trans*-15 18:3	0.01 c	0.01 c	0.02 b	0.09 a	0.008	***	ns	ns
20:2 n-6	0.02 b	0.01 c	0.02 a	0.01 d	0.002	***	**	ns
20:3 n-6	0.02	0.02	0.02	0.02	0.002	ns	***	ns
20:3 n-3	0.01 b	0.01 b	0.01 ab	0.01 a	0.001	*	**	ns
20:4 n-6	0.14 a	0.11 b	0.12 a	0.10 b	0.011	***	**	ns
20:5 n-3	0.04 b	0.03 c	0.03 c	0.05 a	0.004	***	ns	ns
22:2	0.02	0.01	0.01	0.02	0.005	ns	*	ns
22:4	0.02 a	0.02 b	0.02 a	0.01 b	0.003	**	**	ns
22:5 n-3	0.08 b	0.06 c	0.07 bc	0.11 a	0.009	***	*	ns
22:6 n-3	0.02 b	0.02 b	0.02 b	0.04 a	0.004	***	ns	ns
total SFA	76.80 a	66.35 b	63.00 c	65.56 b	1.507	***	ns	ns
total MUFA	19.38 c	29.98 b	29.44 b	26.62 a	1.230	***	ns	ns
total PUFA	3.82 b	3.68 b	7.57 a	7.82 a	0.461	***	*	ns
total conjugated 18:2	0.46 d	0.97 c	2.71 a	1.97 b	0.257	***	ns	ns
total n-6	2.21 b	1.66 c	3.36 a	1.91 c	0.188	***	*	ns
total n-3	0.67 b	0.48 c	0.66 b	1.28 a	0.071	***	**	ns
n-6/n-3	3.34 b	3.50 b	5.13 a	1.53 c	0.193	***	ns	ns
atherogenicity index	3.39 a	1.95 b	1.71 c	1.88 bc	0.155	***	ns	ns

[a] a,b,c,d: Different letters indicate significant differences ($P < 0.05$). P value: *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; ns = $P > 0.05$. MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid; SED = standard error of difference. n-3 = 18:3 n-3 + 20:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3. n-6 = *cis*-9 *cis*-12 18:2 + 18:3 n-6 + 20:2 n-6 + 20:3 n-6 + 20:4 n-6. Atherogenicity index = (12:0 + (4 × (14:0) + 16:0)/(MUFA + PUFA).

α-linolenic acid, **Table 1**) would be completely biohydrogenated to form stearic acid in the rumen (*21*). Oleic acid was the prevailing monounsaturated FA observed in milk fat from all treatments. The highest concentration of oleic acid was reached in the OO diet, with enhancements of more than 48% in relation to control. These results are on the one hand, a consequence of the higher oleic acid presence in the ration and, on the other, due to the action of the Δ-9 desaturase enzyme in the mammary gland (*22*).

The levels of *trans* monounsaturated FAs were increased in all diets including oils with unsaturated FAs (**Figure 1**). The highest concentrations of *trans* (6 + 7 + 8) and *trans*-9 C18:1 in milk fat were obtained with OO supplementation ($P < 0.001$; **Table 3**). This effect, together with the high correlation observed between them ($P < 0.001$; $R^2 = 0.98$), would be an evidence of the extensive oleic acid isomerization by rumen microorganisms. As previously observed in in vitro experiments (*23*) and in ewes fed high amounts of OO (*14*), oleic acid rumen metabolism could involve the formation of several positional *trans* isomers which could afterward be transferred to milk fat.

Trans-10 C18:1 levels were maintained below 1% of total FAME in milk fat throughout all the experimental treatments (**Table 3**; **Figure 1**), ranging from percentages of 0.27 (control), to 0.45 (LO), 0.77 (SO), and 0.79 (OO), suggesting that this FA is produced mainly by oleic and linoleic acids biohydrogenation in the rumen. Previous reports have associated high contents of *trans*-10 C18:1 in dairy fat with alterations in rumen environment, most likely due to diets low in forage or highly supplemented with polyunsaturated FAs (*6*). In dairy ewe, diets with a high proportion of concentrate in presence of 6% of olive (*14*), sunflower (*15*), or soybean (*16*) oils produced levels of *trans*-10 C18:1 that accounted for 3.9, 4.9, and 6.1 g/100 g of total FAME, respectively. Our results indicate that adding 3% of vegetable oil to a 50:50 forage:concentrate diet would scarcely alter rumen lipid metabolism in ewes.

In contrast to *trans*-10 C18:1, VA concentrations, when compared with the control, were multiplied by 2.7, 5.5, and 8.4 in ewes fed rations supplemented with 3% of OO, LO, and SO, respectively (**Table 3**; **Figure 1**). The lowest VA percentage found in the control treatment must be due to the minimal content of unsaturated FAs in the lipid supplement. Under conditions of normal rumen behavior, VA is the main *trans* monounsaturated FA produced during polyunsaturated FA biohydrogenation in dairy ruminants (*2*, *24*). The lower VA levels observed in OO treatment, when compared with SO and LO, could be justified if we bear in mind that this *trans* FA is not the only *trans* monounsaturated FA generated during oleic acid rumen biohydrogenation (*23*). Conversely, supplements rich in linoleic (SO) and α-linolenic (LO) acids were the most efficient at increasing milk VA because both FAs are preferentially converted to this *trans* monounsaturated FA in the rumen (*21*).

Under the experimental conditions assayed, the RA content of milk fat rose from 0.39 (control) to 0.91 (OO), 1.59 (LO), and 2.58 (SO) (**Table 3**). RA is formed not only by direct isomerization of

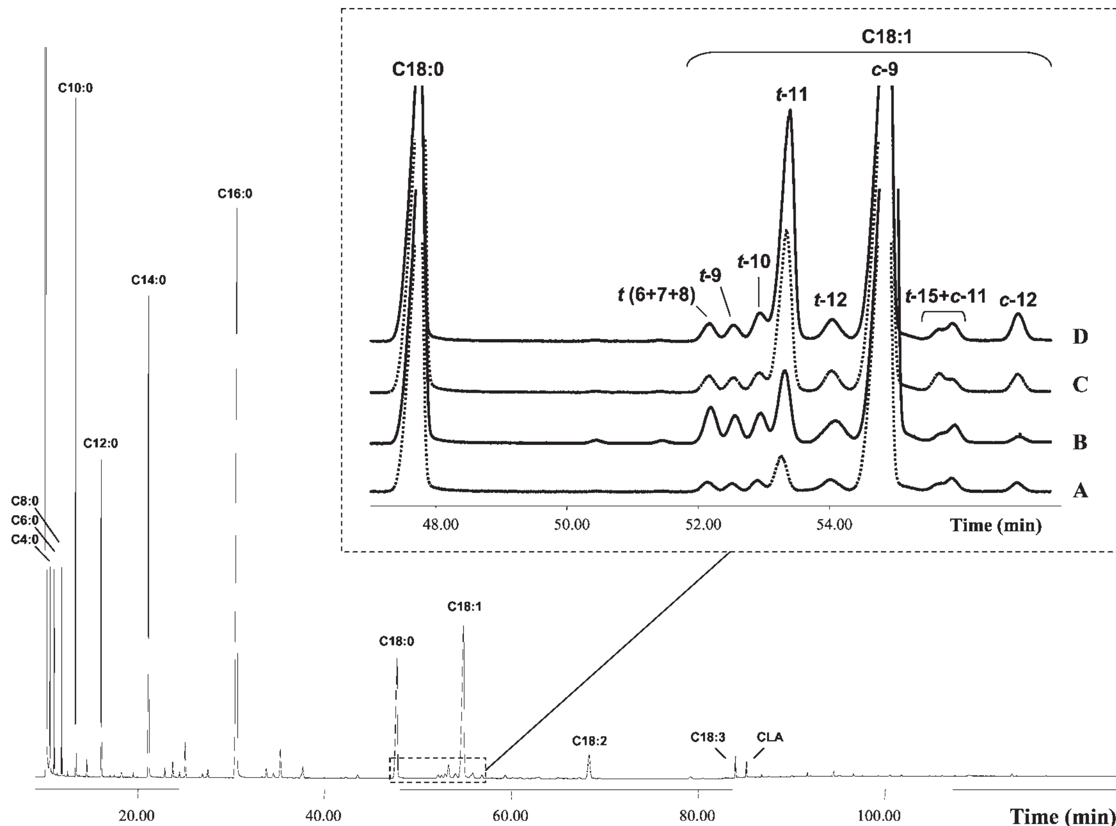

Figure 1. Gas chromatogram of fatty acid methyl esters (FAME) from ewes milk fed a diet supplemented with 3% of palm oil. Expanded area shows partial gas chromatograms from C18:0 to *cis*-12 C18:1 region of the sample above (3% palm oil, A), as well as FAME from ewes milk fat fed diets supplemented with 3% of olive (B), linseed (C), and sunflower (D) oils. *c*: *cis*; *t*: *trans*.

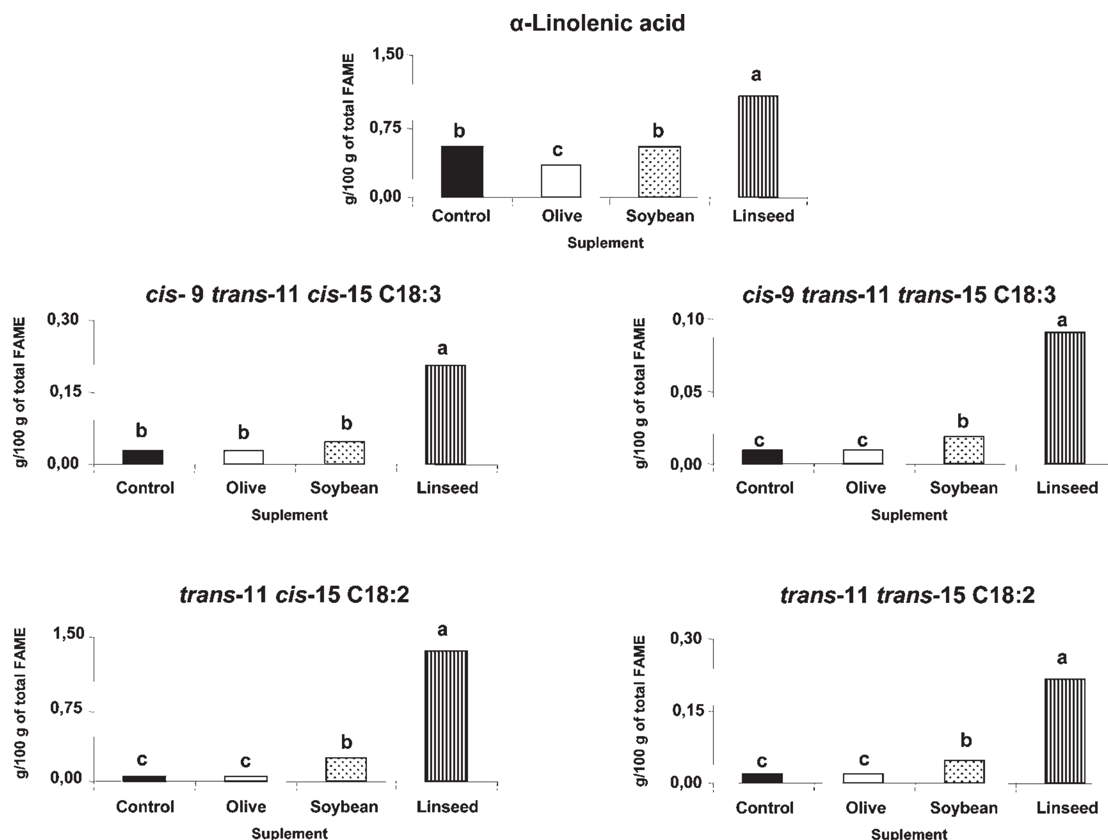

Figure 2. Mean values of α-linolenic acid, *cis*-9 *trans*-11 *cis*-15 C18:3, *cis*-9 *trans*-11 *trans*-15 C18:3, *trans*-11 *cis*-15 C18:2, and *trans*-11 *trans*-15 C18:2 in milk fat of ewes fed the different supplements assayed in the present study. a,b,c: Different letters indicate significant differences between treatments ($P < 0.05$).

linoleic acid in the rumen but mainly by VA desaturation in different tissues (6). Castro et al. (25) supplemented a similar basal diet with less SO (1.2%) and by so doing slightly modified RA concentration in ewe milk fat. A more elevated proportion of this CLA isomer in milk, around 3.5 g/100 g of total FAME, was reached when a 6% of soybean (16) or sunflower (15) oil was

Table 4. Fatty Acid Profile (g/100 g of Total Fatty Acid Methyl Esters) of Raw Milk and Cheese Fat from Ewes Fed with Supplements (3% of DM) of Palm (Control), Olive (OO), Soybean (SO), and Linseed (LO) Oils[a]

	treatment				sample			*P* value		
	control	OO	SO	LO	milk	cheese	SED	T	S	T × S
					SFA					
4:0	3.80 c	3.79 c	3.99 b	4.14 a	3.91	3.95	0.064	***	ns	ns
6:0	3.39 a	2.78 c	2.57 d	3.06 b	2.95	2.95	0.037	***	ns	ns
8:0	3.20 a	2.35 c	2.04 d	2.58 b	2.56	2.52	0.036	***	ns	ns
10:0	9.98 a	6.54 c	5.49 d	6.89 b	7.27	7.18	0.140	***	ns	ns
12:0	5.40 a	3.52 b	3.04 c	3.56 b	3.89	3.87	0.103	***	ns	ns
13:0 *iso*	0.03 a	0.02 b	0.02 b	0.02 b	0.02	0.02	0.001	***	ns	ns
13:0 *anteiso*	0.05 a	0.04 b	0.03 c	0.03 bc	0.04	0.04	0.003	***	ns	ns
13:0	0.18 a	0.11 b	0.10 c	0.10 bc	0.12	0.12	0.005	***	ns	ns
14:0 *iso*	0.11 a	0.08 c	0.09 b	0.08 c	0.09	0.09	0.004	***	ns	ns
14:0	11.37 a	9.52 b	8.74 c	9.52 b	9.79	9.79	0.117	***	ns	ns
15:0 *iso*	0.26 a	0.21 c	0.22 b	0.21 c	0.22	0.23	0.005	***	ns	ns
15:0 *anteiso*	0.42 a	0.29 d	0.35 b	0.31 c	0.34	0.34	0.008	***	ns	ns
15:0	0.96 a	0.72 bc	0.74 b	0.70 c	0.78	0.78	0.014	***	ns	ns
16:0 *iso*	0.25 a	0.20 c	0.22 b	0.20 c	0.21	0.22	0.006	***	ns	ns
16:0	28.49 a	23.06 b	22.47 c	21.61 d	23.87	23.94	0.137	***	ns	ns
17:0	0.51 a	0.40 c	0.44 b	0.44 b	0.45	0.45	0.011	***	ns	ns
18:0 *iso*	0.07 a	0.05 b	0.06 a	0.05 b	0.05	0.06	0.003	***	ns	ns
18:0	8.60 c	11.79 a	10.94 b	11.80 a	10.73	10.83	0.314	***	ns	ns
19:0	0.09 a	0.07 b	0.06 c	0.05 c	0.06	0.07	0.005	***	ns	ns
20:0	0.21 c	0.23 b	0.24 a	0.19 d	0.21	0.22	0.005	***	*	ns
21:0	0.07	0.06	0.06	0.09	0.06	0.08	0.021	ns	ns	ns
22:0	0.10 b	0.09 c	0.14a	0.10 c	0.11	0.11	0.003	***	ns	ns
23:0	0.08 a	0.06 b	0.06b	0.06 b	0.07	0.06	0.005	***	ns	ns
24:0	0.05 a	0.04 bc	0.04ab	0.04 c	0.04	0.04	0.003	*	ns	ns
					MUFA					
10:1	0.38 a	0.25 b	0.20c	0.23b	0.27	0.26	0.009	***	ns	ns
cis-9 14:1	0.17 a	0.16 a	0.14 b	0.13c	0.15	0.15	0.006	***	ns	ns
15:1	0.13 a	0.10 b	0.10 b	0.10b	0.10	0.11	0.003	***	ns	ns
trans-9 16:1 + 17:0 *iso*	0.39 c	0.39 c	0.74 a	0.56b	0.52	0.52	0.011	***	ns	ns
cis-7 16:1	0.26 b	0.32 a	0.26 b	0.27b	0.28	0.28	0.005	***	ns	ns
cis-9 16:1 + 17:0 *anteiso*	1.18 a	1.00 b	0.94 c	0.82d	0.99	0.98	0.016	***	ns	ns
cis-13 16:1	0.11 a	0.06 b	0.04 c	0.05 b	0.07	0.06	0.005	***	ns	ns
17:1	0.18 a	0.15 b	0.14 c	0.12 d	0.14	0.14	0.005	***	ns	ns
trans (6 + 7+8) 18:1	0.27 d	1.07 a	0.54 b	0.47 c	0.59	0.58	0.014	***	ns	ns
trans-9 18:1	0.22 d	0.80 a	0.48 b	0.40 c	0.47	0.48	0.016	***	ns	ns
trans-10 18:1	0.28 c	0.85 a	0.89 a	0.53 b	0.63	0.65	0.036	***	ns	ns
trans-11 18:1 (VA)	0.91 d	2.08 c	6.98 a	4.58 b	3.64	3.63	0.132	***	ns	ns
trans-12 18:1	0.32 d	0.95 a	0.72 b	0.68 c	0.67	0.67	0.016	***	ns	ns
cis-9 18:1	12.62 c	20.74 a	15.86 b	15.51 b	16.27	16.10	0.241	***	ns	ns
cis-11 + *trans*-15 18:1	0.42 c	0.63 b	0.72 a	0.74 a	0.63	0.63	0.009	***	ns	ns
cis-12 18:1	0.21 c	0.17 c	0.84 a	0.52 b	0.44	0.44	0.023	***	ns	ns
cis-13 18:1	0.06 b	0.06 b	0.08 a	0.07 a	0.07	0.07	0.005	**	ns	ns
cis-14 + *trans*-16 18:1	0.28 c	0.27 c	0.49 b	0.64 a	0.42	0.42	0.053	***	ns	ns
cis-15 18:1	0.08 b	0.18 b	0.14 b	0.33 a	0.18	0.18	0.050	**	ns	ns
cis-16 18:1	0.04 b	0.04 b	0.03 c	0.06 a	0.04	0.04	0.004	***	ns	ns
cis-9 20:1	0.02 c	0.06 a	0.05 a	0.04 b	0.04	0.04	0.003	***	ns	ns
24:1	0.02	0.02	0.02	0.02	0.02	0.02	0.003	ns	ns	ns
					Nonconjugated 18:2					
trans-11 *trans*-15	0.03 c	0.04 c	0.06 b	0.23 a	0.09	0.09	0.008	***	ns	ns
trans-9 *trans*-12	0.06 d	0.07 c	0.10 b	0.12 a	0.08	0.09	0.007	***	ns	ns
trans-8 *cis*-12 + *cis*-9 *trans*-13	0.09 d	0.12 c	0.16 b	0.22 a	0.15	0.15	0.008	***	ns	ns
trans-8 *cis*-13	0.08 c	0.09 bc	0.09 b	0.15 a	0.10	0.10	0.006	***	ns	ns
cis-9 *trans*-12	0.03 b	0.04 a	0.03 b	0.04 a	0.04	0.03	0.005	*	ns	ns
trans-9 *cis*-12	0.03 b	0.04 b	0.05 b	0.07 a	0.05	0.04	0.009	**	ns	ns
trans-11 *cis*-15	0.11 c	0.07 c	0.22 b	1.15 a	0.39	0.39	0.021	***	ns	ns
cis-9 *cis*-12	1.83 b	1.46 d	2.93 a	1.58 c	1.93	1.96	0.047	***	ns	ns
other 18:2	0.06 c	0.07 c	0.09 b	0.10 a	0.08	0.08	0.005	***	ns	ns

Table 4. Continued

	treatment				sample			*P* value		
	control	OO	SO	LO	milk	cheese	SED	T	S	T × S
				Conjugated 18:2						
cis-9 *trans*-11 (RA)	0.43 d	0.92 c	2.71 a	1.64 b	1.40	1.45	0.053	***	ns	ns
trans-9 *cis*-11	0.01 b	0.02 a	0.02 a	0.01 b	0.01	0.01	0.002	**	ns	ns
trans-10 *cis*-12	0.01	0.01	0.01	0.01	0.01	0.01	0.002	ns	ns	ns
trans-11 *cis*-13	0.02 b	0.01 b	0.03 b	0.18 a	0.07	0.05	0.025	***	ns	ns
trans-12 *trans*-14	0.02 b	0.01 c	0.02 b	0.07 a	0.03	0.03	0.002	***	ns	ns
trans-11 *trans*-13	0.04 b	0.02 c	0.03 bc	0.08 a	0.04	0.04	0.007	***	ns	ns
other *trans*−*trans*	0.01 c	0.01 c	0.04 a	0.02 b	0.02	0.02	0.003	***	ns	ns
				Other PUFA						
18:3 n-6	0.05 a	0.03 b	0.03 b	0.02 c	0.03	0.03	0.003	***	ns	ns
18:3 n-3	0.54 b	0.36 c	0.51 b	1.04 a	0.61	0.61	0.014	***	ns	ns
cis-9 *trans*-11 *cis*-15 18:3	0.04 b	0.04 c	0.04 b	0.19 a	0.08	0.08	0.003	***	ns	ns
cis-9 *trans*-11 *trans*-15 18:3	0.01 c	0.01 c	0.02 b	0.09 a	0.03	0.03	0.002	***	ns	ns
20:2 n-6	0.02	0.01	0.02	0.03	0.02	0.03	0.010	ns	ns	ns
20:3 n-6	0.02 a	0.02 a	0.02 a	0.01 b	0.02	0.02	0.002	**	ns	ns
20:3 n-3	0.01	0.01	0.01	0.01	0.01	0.01	0.002	ns	ns	ns
20:4 n-6	0.14 a	0.12 a	0.13 a	0.08 b	0.11	0.12	0.012	**	ns	ns
20:5 n-3	0.04	0.03	0.03	0.03	0.04	0.03	0.006	ns	ns	ns
22:2	0.02 b	0.01 b	0.01 b	0.04 a	0.02	0.02	0.007	**	ns	ns
22:4	0.02 a	0.02 a	0.02 a	0.01 b	0.02	0.02	0.002	***	ns	ns
22:5 n-3	0.07 b	0.06 c	0.06 c	0.09 a	0.07	0.07	0.003	***	ns	ns
22:6 n-3	0.02 bc	0.03 b	0.02 c	0.03 a	0.02	0.03	0.002	***	ns	ns
total SFA	78.02 a	66.22 b	62.34 c	66.05 b	68.12	68.20	0.309	***	ns	ns
total MUFA	18.13 c	30.04 a	30.14 a	26.57 b	26.30	26.14	0.282	***	ns	ns
total PUFA	3.85 b	3.74 b	7.52 a	7.38 a	5.58	5.66	0.071	***	ns	ns
total conjugated 18:2	0.53 d	1.00 c	2.86 a	2.01 b	1.59	1.61	0.062	***	ns	ns
total n-6	2.06 b	1.63 c	3.12 a	1.72 c	2.11	2.16	0.050	***	ns	ns
total n-3	0.69 b	0.48 d	0.64 c	1.21 a	0.75	0.75	0.016	***	ns	ns
n-6/n-3	3.00 c	3.38 b	4.90 a	1.43 d	3.15	3.20	0.092	***	ns	ns
atherogenicity index	3.61 a	1.91 b	1.61 c	1.86 b	2.25	2.24	0.051	***	ns	ns

[a] a,b,c,d: Different letters indicate significant differences ($P < 0.05$). *P* value: effects due to treatment (T), sample (S), and their interaction (T × S); *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; ns = P > 0.05. MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid; SED = standard error of difference. n-3 = 18:3 n-3 + 20:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3. n-6 = *cis*-9 *cis*-12 18:2 + 18:3 n-6 + 20:2 n-6 + 20:3 n-6 + 20:4 n-6. Atherogenicity index = (12:0 + (4 × (14:0) + 16:0)/(MUFA + PUFA).

incorporated to the ration. The higher levels of RA obtained with SO, relative to LO ($P < 0.001$; **Table 3**), can be attributed to the linoleic acid levels present in the rations. Linoleic acid is directly isomerized to form RA in the rumen besides its endogenous synthesis via Δ-9 desaturase. However, α-linolenic acid can only be transformed into RA by desaturation in the mammary gland from VA generated by ruminal biohydrogenation (*21*).

Trans-10 *cis*-12 and *trans*-9 *cis*-11 C18:2 amounts were negligible in all diets (**Table 3**). These FAs have been associated with changes in rumen lipid metabolism and could contribute toward inhibiting milk fat synthesis in the udder (*26*). All the experimental treatments assayed in the current study presented low levels for both CLA isomers and *trans*-10 C18:1, which implies an unmodified rumen function and no shift in the unsaturated FA biohydrogenation pathways.

Other CLA isomers, *trans*-11 *cis*-13, *trans*-11 *trans*-13, and *trans*-12 *trans*-14 increased significantly ($P < 0.001$) in milk fat from ewes fed LO diet (**Table 3**), as formerly reported in ruminants fed on pasture (*27,28*) or with diets rich in linseed (*29*−*31*). This enhancement could be due to the presence of α-linolenic acid in the rations, but the precursors and reactions involved in their metabolism are far from clear.

Supplementation with SO increased linoleic acid concentration in dairy fat ($P < 0.001$), reaching similar values (>3% of total FAME) to those obtained with higher amounts of SO (*15*). In contrast, levels of this essential n-6 FA were hardly modified by the rest of the experimental rations (**Table 3**) because of their lower content of linoleic acid. The response to feeding 3% of LO was marked by the presence of α-linolenic acid and its rumen conventional intermediates: rumelenic acid (*cis*-9 *trans*-11 *cis*-15 C18:3), *trans*-11 *cis*-15 C18:2, VA, and *cis*-15 C18:1 ($P < 0.001$; **Table 3**) (*21*). Gómez-Cortés et al. (*32*) have recently postulated another possible biohydrogenation pathway for α-linolenic acid from rumelenic acid. This FA could be isomerized to *cis*-9 *trans*-11 *trans*-15 C18:3 and then hydrogenated to form *trans*-11 *trans*-15 C18:2, VA, and *trans*-15 C18:1. The incorporation of 3% of LO in dairy ewe diets significantly augmented C18:3 biohydrogenation intermediates ($P < 0.001$, **Table 3**), but those increases were not observed with the addition of other plant oils (**Figure 2**). Our results would support all these ruminal biohydrogenation pathways for α-linolenic acid.

Cheese Fatty Acid Profile. As can be appreciated in **Table 4**, cheesemaking and ripening did not modify the FA profile of dairy fats. It has been stated that the effect of cheese manufacture on its composition is minimal compared to the many variations associated with diet formulations for ruminants (*33*), although noticeable changes could occur in the CLA positional and geometrical isomers during cheese ripening. Recently RA content was enhanced by about 10% with aging (*34*), but further studies would be necessary to determine more precisely the role of starter cultures in the distribution pattern of CLA isomers during the ripening of cheese. However, from a quantitative point of view,

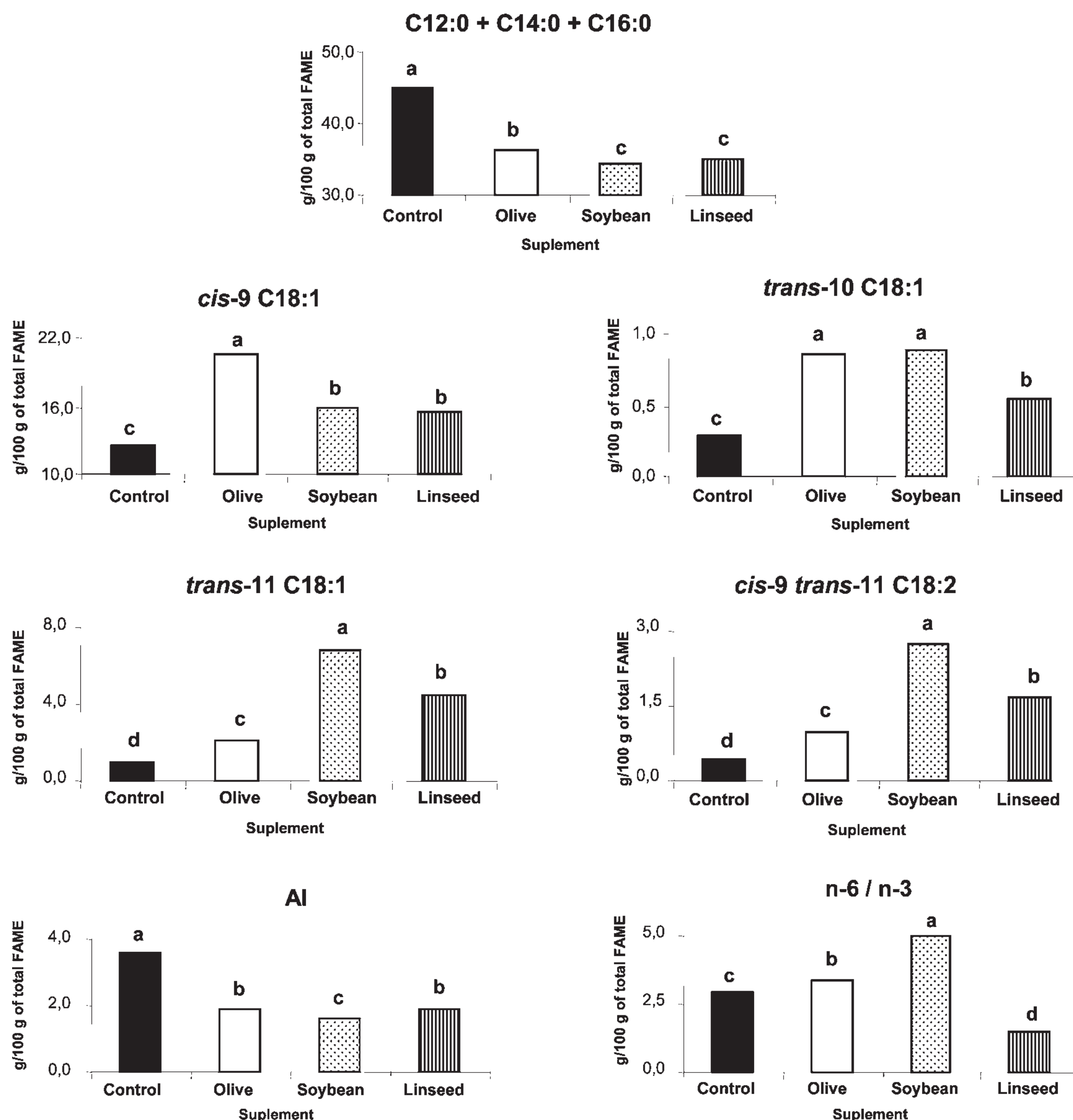

Figure 3. Mean values of C12:0 + C14:0 + C16:0, *cis*-9 C18:1, *trans*-10 C18:1, *trans*-11 C18:1, *cis*-9 *trans*-11 C18.2, atherogenicity index (AI), and n-6/n-3 ratio in cheese made from ewes milk fed the different supplements assayed in the present study. a,b,c,d; Different letters indicate significant differences between treatments ($P < 0.05$).

the most suitable strategy for improving CLA and n-3 FAs in ewe cheeses is by enhancing their concentrations in raw milk (*31*, *35*).

Consumption of dairy products is not always recommended because they contain substantial amounts of saturated FAs. However, in this respect, one of the encouraging results observed in the present study was the sharp decrease in C12:0, C14:0, and C16:0 levels in cheeses made from milk of ewes fed rations supplemented with 3% of OO, SO, and LO (**Table 4**; **Figure 3**). The reduction obtained was on average 23% compared with control cheese. As a result of such decreases coupled with increases in most of the monounsaturated and polyunsaturated FAs, the atherogenicity index value (IA) in OO, SO, and LO cheeses dropped in the middle as was previously reported (*31*). Thus the nutritional value of these cheeses was improved because the IA is a parameter related to the risk of atherosclerosis.

The amount of *trans* FAs in the human diet is also of interest because of the possible adverse effects these isomers have on cardiovascular disease, infant development, diabetes, and inflammation (*36*). Nevertheless, consumers should be aware of the isomer-specific effects of *trans* FAs. Two specific isomers are of particular interest: *trans*-10 C18:1, which has been linked with increases in triglyceride levels, the LDL/HDL ratio, and aortic lipid deposition in animal models, and VA, a FA that may offer some protection against cardiovascular heart disease (*37*−*39*). Taking into account the foregoing, it should be noted that the *trans* C18:1 profile in all experimental cheeses was positive from a nutritional standpoint. While VA reached values of up to 4.6% and 7.0% of total FAME with LO and SO diets, *trans*-10 C18:1 levels were always lower than 1% of total FAME (**Table 4**; **Figure 3**). Moreover, when compared with other unsaturated lipid supplements, *trans*-10 C18:1 concentrations were distinctively the lowest in LO cheeses (0.5%).

The balance of n-6/n-3 FAs is also an important determinant in decreasing the risk of coronary heart disease because it favors the endogenous synthesis of long chain n-3 FAs (*40*). There is still some controversy about the optimal n-6/n-3 coefficient because it depends on the disease under consideration. However, in contrast to SO, the lowest ratio observed in LO treatment (<1.5, **Table 4**; **Figure 3**) suggests that LO cheeses could be an advisable nutritional option for improving the FA profile in cheese and milk from ewes.

A dairy ewe diet with similar proportions of forage and concentrate supplemented with 3% of plant oil could be a valuable procedure for improving cheese and milk quality from a nutritional point of view. C12:0 + C14:0 + C16:0 levels were reduced on average by 23% with the inclusion of OO, SO, and LO when compared with the control. Moreover, the *trans* FA profiles were acceptable in all experimental treatments, mainly due to their low *trans*-10 C18:1 concentrations. The addition of LO and SO was more effective at enriching VA and RA in cheese and milk from ewes than OO or hydrogenated palm oil. However, the significant drop in the n-6/n-3 ratio observed with LO cheese suggests that such lipid supplementation in dairy ewe diets would be the most effective nutritional strategy for improving the milk FA profile in dairy products.

ABBREVIATIONS USED

AI, atherogenicity index; BW, body weight; CLA, conjugated linoleic acid; DM, dry matter; FA, fatty acid; FAME, fatty acid methyl esters; LO, linseed oil; MUFA, monounsaturated fatty acid; OO, olive oil; PUFA, polyunsaturated fatty acid; RA, rumenic acid; SED, standard error of difference; SFA, saturated fatty acid; SO, soybean oil; TMR, total mixed ration; VA, vaccenic acid.

ACKNOWLEDGMENT

We thank M.V. Rodríguez-Pino for her valuable technical assistance.

LITERATURE CITED

(1) Sanz Sampelayo, M. R.; Chilliard, Y.; Schmidely, P.; Boza, J. Influence of type of diet on the fat constituents of goat and sheep milk. *Small Ruminant Res.* **2007**, *68*, 42–63.

(2) Chilliard, Y.; Ferlay, A. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reprod. Nutr. Dev.* **2004**, *44*, 467–492.

(3) Lock, A. L.; Kraft, J.; Rice, B. H.; Bauman, D. E. Biosynthesis and biological activity of rumenic acid: a natural CLA isomer. In *Trans Fatty Acids in Human Nutrition*; Destaillats, F., Sébédio, J. L., Dionisi, F., Chardigny, J. M., Eds.; The Oily Press Lipid Library: Bridgwater, UK, 2009; pp 195−230.

(4) Chilliard, Y.; Martin, C.; Rouel, J.; Doreau, M. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. *J. Dairy Sci.* **2009**, *92*, 5199–5211.

(5) Doreau, M.; Laverroux, S.; Normand, J.; Chesneau, G.; Glasser, F. Effect of linseed fed as rolled seeds, extruded seeds or oil on fatty acid rumen metabolism and intestinal digestibility in cows. *Lipids* **2009**, *44*, 53–62.

(6) Palmquist, D. L.; Lock, A. L.; Shingfield, K. J.; Bauman, D. E. Biosynthesis of conjugated linoleic acid in ruminants and humans. In *Advances in Food and Nutrition Research*; Taylor, S. L., Ed.; Elsevier Inc: San Diego, CA, 2005; Vol. 50, pp 179−217.

(7) Juárez, M.; Ramos, M. Sheep Milk. In *Encyclopedia of Dairy Sciences*; Roginski, H., Fuquay, J. W., Fox, P. F., Eds.; Academic Press: United Kingdom, 2003; Vol. 4, pp 2539−2545.

(8) Association of Official Analytical Chemistry. *Official Methods of Analysis*, 17th ed.; Association of Official Analytical Chemistry: Gaithersburg, MD, 2003.

(9) IDF. *Determination of Milk Fat, Protein and Lactose Content. Guidance on the Operation of Mid-Infrared Instruments*; International IDF standard 141c:2000, 2000.

(10) Luna, P.; Juárez, M.; De la Fuente, M. A. Validation of a rapid milk fat separation method to determine the fatty acid profile by gas chromatography. *J. Dairy Sci.* **2005**, *88*, 3377–3381.

(11) ISO-IDF. *Milk and Milk Products—Extraction Methods for Lipids and Liposoluble Compounds*; International Standard ISO 14156-IDF 172:2001, 2001.

(12) ISO-IDF. *Milk Fat—Preparation of Fatty Acid Methyl Esters*; International Standard ISO 15884-IDF 182:2002, 2002.

(13) ISO-IDF. *Milk Fat. Determination of the Fatty Acid Composition by Gas-Liquid Chromatography*; International Standard ISO 15885-IDF 184:2002(E), 2002.

(14) Gómez-Cortés, P.; Frutos, P.; Mantecón, A. R.; Juárez, M.; De la Fuente, M. A.; Hervás, G. Addition of olive oil to dairy ewe diet: effect on milk fatty acid profile and animal performance. *J. Dairy Sci.* **2008**, *91*, 3119–3127.

(15) Hervás, G.; Luna, P.; Mantecón, A. R.; Castañares, N.; De la Fuente, M. A.; Juárez, M.; Frutos, P. Effect of diet supplementation with sunflower oil on milk production, fatty acid profile and rumen fermentation. *J. Dairy Res.* **2008**, *75*, 399–405.

(16) Gómez-Cortés, P.; Frutos, P.; Mantecón, A. R.; Juárez, M.; De la Fuente, M. A.; Hervás, G. Milk production, conjugated linoleic acid content, and in vitro ruminal fermentation in response to high levels of soybean oil in dairy ewe diet. *J. Dairy Sci.* **2008**, *91*, 1560–1569.

(17) Zhang, R. H.; Mustafa, A. F.; Zhao, X. Blood metabolites and fatty acid composition of milk and cheese from ewes fed oilseeds. *Can. J. Anim. Sci.* **2006**, *86*, 547–556.

(18) Zhang, R.; Mustafa, A.; Zhao, X. Effects of flaxseed supplementation to lactating ewes on milk composition, cheese yield, and fatty acid composition of milk and cheese. *Small Ruminant Res.* **2006**, *63*, 233–241.

(19) Zhang, R. H.; Mustafa, A. F.; Zhao, X. Effects of feeding oilseeds rich in linoleic and linolenic fatty acids to lactating ewes on cheese yield and on fatty acid composition of milk and cheese. *Anim. Feed Sci. Technol.* **2006**, *127*, 220–233.

(20) Kadegowda, A. K. G.; Bionaz, M.; Piperova, L. S.; Erdman, R. A.; Loor, J. J. Peroxisome proliferators-activated receptor-γ activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents. *J. Dairy Sci.* **2009**, *92*, 4276–4279.

(21) Harfoot, C. G.; Hazlewood, G. P. Lipid metabolism in the rumen. In *The Rumen Microbial Ecosystem*; Hobson, P. N., Stewart, C. S., Eds.; Blackie Academic & Professional: New York, 1997; pp 382−426.

(22) Lock, A. L.; Garnsworthy, P. C. Seasonal variation in milk conjugated linoleic acid and delta-9 desaturase in dairy cows. *Livestock Prod. Sci.* **2003**, *79*, 47–59.

(23) Mosley, E. E.; Powell, G. L.; Riley, M. B.; Jenkins, T. C. Microbial biohydrogenation of oleic acid to *trans* isomers in vitro. *J. Lipid Res.* **2002**, *43*, 290–296.

(24) Shingfield, K. J.; Chilliard, Y.; Toivonen, V.; Kairenius, P.; Givens, D. I. *Trans* fatty acids and bioactive lipids in ruminant milk. *Adv. Exp. Med. Biol.* **2008**, *606*, 3–65.

(25) Castro, T.; Manso, T.; Jimeno, V.; Del Alamo, M.; Mantecón, A. R. Effects of dietary sources of vegetable fats on performance of dairy ewes and conjugated linoleic acid (CLA) in milk. *Small Ruminant Res.* **2009**, *84*, 47–53.

(26) Shingfield, K. J.; Griinari, J. M. Role of biohydrogenation intermediates in milk fat depression. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 799–816.

(27) Gómez-Cortés, P.; Frutos, P.; Mantecón, A. R.; Juárez, M.; De la Fuente, M. A.; Hervás, G. Effect of the supplementation of grazing dairy ewes with a cereal concentrate on animal performance, milk fatty acid profile and rumen fermentation. *J. Dairy Sci.* **2009**, *92*, 3964–3972.

(28) Kraft, J.; Collomb, M.; Möckel, P.; Sieber, R.; Jahreis, G. Differences in CLA isomer distribution of cows' milk lipids. *Lipids* **2003**, *38*, 657–664.

(29) Collomb, M.; Sieber, R.; Bütikofer, U. CLA isomers in milk fat from cows fed diets with high levels of unsaturated fatty acids. *Lipids* **2004**, *39*, 355–364.

(30) Luna, P.; Fontecha, J.; Juárez, M.; De la Fuente, M. A. Changes in the milk and cheese fat composition of ewes fed commercial supplements containing linseed with special reference to the CLA content and isomer composition. *Lipids* **2005**, *40*, 445–454.

(31) Gómez-Cortés, P.; Bach, A.; Luna, P.; Juárez, M.; De la Fuente, M. A. Extruded Linseed on Ewes Rations Improves Omega-3 Fatty Acids and Conjugated Linoleic Acid Contents in Cheese and Milk Fat. *J. Dairy Sci.* **2009**, *92*, 4122–4134.

(32) Gómez-Cortés, P.; Tyburczy, C.; Brenna, J. T.; Juárez, M.; De la Fuente, M. A. Characterization by GC-MS and CACI-MS/MS of *cis*-9 *trans*-11 *trans*-15 C18:3 in milk fat. *J. Lipid Res*. **2009**, *50*, 2412–2420.
(33) Parodi, P. W. Conjugated linoleic acid in food. In *Advances in CLA Research*; Sebedio, J. L., Christie, W. W., Adlof, R., Eds.; AOCS Press: Champaign, IL, 2003; pp 101−122.
(34) Buccioni, A.; Rapaccini, S.; Antongiovanni, M.; Minieri, S.; Conte, G.; Mele, M. Conjugated linoleic acid and C18:1 isomers content in milk fat of sheep and their transfer to Pecorino Toscano cheese. *Int. Dairy J*. **2010**, *20*, 190–194.
(35) Luna, P.; Juárez, M.; De la Fuente, M. A. Conjugated linoleic acid content and isomer distribution during ripening in three varieties of cheeses protected with designation of origin. *Food Chem*. **2007**, *103*, 1465–1472.
(36) Jakobsen, M. U.; Overvad, K. Observational epidemiological studies on intake of *trans* fatty acids and risk of ischaemic heart disease. In *Trans Fatty Acids in Human Nutrition*; Destaillats, F., Sébédio, J. L., Dionisi, F., Chardigny, J. M., Eds.; The Oily Press Lipid Library: Bridgwater, UK, 2009; pp 255−306.
(37) Roy, A.; Chardigny, J. M.; Bauchart, D.; Ferlay, A.; Lorenz, S.; Durand, D.; Gruffat, D.; Faulconnier, Y.; Sébédio, J. L.; Chilliard, Y. Butters rich either in *trans*-10-C18:1 or in *trans*-11-C18:1 plus *cis*-9, *trans*-11 CLA differentially affect plasma lipids and aortic fatty streak in experimental atherosclerosis in rabbits. *Animal* **2007**, *1*, 467–476.
(38) Anadón, A.; Martínez-Larrañaga, M. R.; Martínez, M. A.; Ares, I.; Ramos, E.; Gómez-Cortés, P.; Juárez, M.; De la Fuente, M. A. Acute Oral Safety Study of Dairy Fat Rich in *trans*-10 C18:1 versus Vaccenic plus Conjugated Linoleic Acid in Rats. *Food Chem. Toxicol*. **2010**, *48*, 591–598.
(39) Bauchart, D.; Roy, A.; Lorenz, S.; Chardigny, J. M.; Ferlay, A.; Gruffat, D.; Sébédio, J. L.; Chilliard, Y.; Durand, D. Butters varying in *trans* 18:1 and *cis*-9, *trans*-11 conjugated linoleic acid modify plasma lipoproteins in the hypercholesterolemic rabbit. *Lipids* **2007**, *42*, 123–133.
(40) Simopoulos, A. P. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp. Biol. Med*. **2008**, *233*, 674–688.

Received for review May 7, 2010. Revised manuscript received July 26, 2010. Accepted July 30, 2010. This work was carried out through a collaboration agreement between the Diputación de Palencia and the Universidad de Valladolid and has been subsidized by the Instituto Tecnológico Agrario de Castilla y León (Project VA C2 C), the Consejería de Educación de la Junta de Castilla y León (Project VA058A07), the Ministerio de Ciencia e Innovación (MICINN; AGL2008-04805 and Consolider Ingenio 2010 Programme; FUN-C-FOOD CSD2007-063) as well as by the Comunidad Autónoma de Madrid (2009-AGR-1469). Raúl Bodas has a JAE-Doc contract from the CSIC under the program "Junta para la Ampliación de Estudios".