Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae

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ABSTRACT

In an attempt to develop strategies for enhancing the nutritional value of sheep milk fat, dairy ewe diet was supplemented with 3 incremental levels of marine algae (MA), in combination with sunflower oil, to evaluate the effects of these marine lipids on milk fatty acid (FA) profile and animal performance. Fifty Assaf ewes in mid lactation were distributed in 10 lots of 5 animals each and allocated to 5 treatments (2 lots per treatment): no lipid supplementation (control) or supplementation with 25 g of sunflower oil/kg of DM plus 0 (SO), 8 (SOMA₁), 16 (SOMA₂), or 24 (SOMA₃) g of MA (56.7% ether extract)/kg of DM. Milk production and composition, including FA profile, were analyzed on d 0, 3, 7, 14, 21, and 28 of treatment. Neither intake nor milk yield were significantly affected by lipid addition, but all MA supplements decreased milk fat content from d 14 onward, reaching a 30% reduction after 28 d on SOMA₃. This milk fat depression might be related not only to the joint action of some putative fat synthesis inhibitors, such as *trans*-9,*cis*-11 C18:2 and probably *trans*-10 C18:1, but also to the limited ability of the mammary gland to maintain a desirable milk fat fluidity, that would have been caused by the noticeable increase in *trans*-C18:1 together with the lowered availability of stearic acid for oleic acid synthesis through Δ^9 -desaturase. Furthermore, all lipid supplements, and mainly MA, reduced the secretion of de novo FA (C6:0–C14:0) without increasing the yield of preformed FA (>C16). Supplementation with sunflower oil plus MA resulted in larger increases in *cis*-9,*trans*-11 C18:2 than those observed with sunflower oil alone, achieving a mean content as high as 3.22% of total FA and representing a more than 7-fold increase compared with the control. Vaccenic acid (*trans*-11 C18:1) was also significantly enhanced (on average +794% in SOMA treatments), as was C22:6 n-3 (DHA) content, although

the transfer efficiency of the latter, from the diets to the milk, was very low (5%). However, the highest levels of MA inclusion (SOMA₂ and SOMA₃) reduced the milk n-6:n-3 ratio, but MA supplements caused an important increase in *trans*-10 C18:1, which would rule out the possibility that this milk has a healthier fat profile before determining the specific role of each individual FA and ensuring that this *trans*-FA is at least innocuous in relation to cardiovascular disease risk.

Key words: conjugated linoleic acid, milk fat depression, n-3, *trans* fatty acid

INTRODUCTION

A growing epidemic of chronic disease related to dietary and lifestyle changes afflicts both developed and developing countries, with cardiovascular disease, cancer, and diabetes nowadays being among the most important causes of premature death (WHO, 2003). The discovery of potential anticarcinogenic, antiatherosclerotic, and antidiabetic effects of conjugated linoleic acid (CLA; Pariza et al., 2001; Shingfield et al., 2008) and the recognized role in human health of the n-3 fatty acids (FA; Simopoulos, 2008) have led to an increasing number of studies over the past decade seeking to enhance the content of these bioactive compounds in ruminant-derived products, mainly in cow milk. Even though consumption of ovine milk might have several nutritional advantages over bovine milk consumption, such as its higher mineral (e. g., Ca, P, and Mg), and caprylic (C8:0) and capric (C10:0) acid contents and its easier digestibility (Recio et al., 2009), research in ewes is still scarce.

In a previous study in dairy sheep, inclusion in the diet of sunflower oil (SO), rich in linoleic acid, induced a 4-fold increase in milk CLA content (Hervás et al., 2008), presumably through increased ruminal formation of vaccenic acid (VA; *trans*-11 C18:1; Palmquist et al., 2005; Chilliard et al., 2007), which serves as a substrate for endogenous synthesis of the major isomer of CLA, rumenic acid (RA; *cis*-9,*trans*-11 CLA) not only in the ruminant mammary gland but also in some human tissues (Palmquist et al., 2005). The use of SO

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in combination with long-chain n-3 polyunsaturated fatty acids (**PUFA**) of marine lipids, which are inhibitors of the ruminal reduction of *trans*-C18:1 to stearic acid (C18:0; Loor et al., 2005; Or-Rashid et al., 2008), would induce further increases in VA ruminal out-flow and subsequently RA mammary synthesis, as reported in cows (Shingfield et al., 2006; Cruz-Hernández et al., 2007). However, the nutritional strategy of supplying a source of linoleic acid together with marine lipids may also increase some *trans*-C18:1 in ruminant milk fat (Reynolds et al., 2006; Shingfield et al., 2006; Cruz-Hernández et al., 2007), whose potential specific role for human health is still unclear (Shingfield et al., 2008).

In addition to the effects addressed to modify milk FA profile, the inclusion of fish oil in the diet appears to affect animal performance, reducing milk fat content in both dairy ewes (Capper et al., 2007) and cows (Griinari and Bauman, 2006; Cruz-Hernández et al., 2007; Gama et al., 2008). Addition of marine algae (**MA**), on the contrary, has been reported to induce milk fat depression (**MFD**) in cows (Franklin et al., 1999; Offer et al., 2001; Boeckaert et al., 2008) but not in sheep (Papadopoulos et al., 2002; Reynolds et al., 2006) and is responsible for a greater transfer efficiency of long-chain n-3 PUFA into the milk in this species than in cattle (Papadopoulos et al., 2002; Reynolds et al., 2006; Chilliard et al., 2007). Notwithstanding, effects of MA inclusion described in the literature are quite inconsistent and might depend on several factors, such as basal diet composition and algae dosage (Reynolds et al., 2006). This fact, together with the scarcity of published studies on this issue, makes it difficult to establish an appropriate level of MA inclusion in the diet of sheep to obtain a healthier milk FA profile for human consumers, with no detrimental effects on animal performance. The objective of this study was therefore to investigate the effect of the dietary inclusion of incremental levels of MA, in combination with SO, on dairy ewes' performance and milk FA profile.

MATERIALS AND METHODS

Animals, Experimental Diets, and Management

Fifty multiparous Assaf ewes (BW = 84.9 kg; SD = 11.75) in mid lactation (at wk 14 at the beginning of the experiment; SD = 1.0) were stratified according to milk production, BW, days postpartum, and number of lactation, randomly distributed in 10 lots of 5 animals each, and allocated to 5 experimental treatments (2 lots per treatment): no lipid supplementation or supplementation with SO (Carrefour S.A., Spain), either alone or in combination with 3 increasing levels of MA (DHA Gold Animal Feed Ingredient, Martek

Biosciences Corp., Columbia, MD; 567 g of ether extract/kg of DM).

The diets, prepared weekly, consisted of a TMR based on alfalfa hay (particle size >4 cm) and a concentrate (50:50) either without lipid supplementation (control diet; negative control) or supplemented with 25 g of SO/kg of DM plus 0 (SO diet; positive control), 8 (**SOMA₁**), 16 (**SOMA₂**), or 24 (**SOMA₃**) g of MA/kg of DM. The ingredients and chemical composition of the 5 experimental diets, which included molasses to avoid selection of dietary components, are given in Table 1. During a 3-wk adaptation period (before commencing the trial), all animals received the control diet. Clean water and a vitamin-mineral supplement were always available and fresh diets were offered daily ad libitum at 0900 and 1900 h.

The ewes were milked at approximately 0830 and 1830 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain). The experiment lasted for 4 wk and was carried out in accordance with Spanish Royal Decree 1201/2005 for the protection of animals used for experimental purposes.

Measurements, Sample Collection, and Chemical Analyses

Samples of offered and refused diets were collected once a week, stored at −30°C, and then freeze-dried. The DMI was recorded weekly for each experimental lot. Diet samples were analyzed for DM (ISO, 1999a), ash (ISO, 2002a), and CP (ISO, 2005). Neutral detergent fiber and ADF were determined as described by Ankom Technology (Ankom, 2006a,b). Neutral detergent fiber was assayed with sodium sulfite and α-amylase and expressed with residual ash (the latter also for ADF). The content of ether extract in the diets was determined by the Ankom Filter Bag Technology (American Oil Chemists' Society Official Procedure Am 5-04; AOCS, 2008).

Individual milk yield was recorded on d 0, 3, 7, 14, 21, and 28, both at morning and evening milkings. With the same frequency, milk samples for the analysis of fat, protein, and TS were collected from each animal, composited according to morning and evening milk yield, and treated with natamycin. The protein, fat, and TS concentrations were determined by infrared spectrophotometry (ISO, 1999b) using a MilkoScan 255 A/S N (Foss Electric, Hillerød, Denmark).

Milk FA composition was determined in untreated samples from each experimental lot and composited according to individual milk production within day. Milk fat was extracted as described by Luna et al. (2005), and FA methyl esters (**FAME**) were prepared by base-catalyzed methanolysis of the glycerides (ISO, 2002b).

Table 1. Ingredients and chemical composition of the experimental diets¹

Item	Control	SO	SOMA ₁	SOMA ₂	SOMA ₃
Ingredient, g/kg of fresh matter					
Dehydrated alfalfa hay	484	474	470	466	462
Whole corn grain	136	133	131	130	129
Whole barley grain	175	170	169	168	167
Soybean meal	97	95	94	93	92
Beet pulp	49	47	47	47	46
Molasses	37	36	36	36	36
Feed supplement ²	22	21	21	21	21
Sunflower oil ³	0	24	24	24	24
Marine algae ⁴	0	0	8	15	23
Chemical composition, g/kg of DM					
OM	896	900	897	893	899
CP	161	159	158	159	158
NDF	308	304	296	300	293
ADF	198	195	190	191	187
Ether extract	26	50	54	57	63

¹Diets included a TMR without lipid supplementation (control) or supplementation with 25 g of sunflower oil/kg of DM plus 0 (SO), 8 (SOMA₁), 16 (SOMA₂), or 24 (SOMA₃) g of marine algae (56.7% ether extract)/kg of DM.

²INA OV1, Evalis, Madrid, Spain. Contained (g/kg): NaHCO₃ (333), CaCO₃ (311), Ca₂HPO₄ (133), mine salt (111), and mineral and vitamins (111).

³Contained (% total fatty acid methyl esters): C16:0 (7.5), C18:0 (4.3), C18:1 (26.3), and C18:2 (60.5).

⁴As declared by the supplier (Martek Biosciences Corp., Columbia, MD), marine algae (DHA Gold Animal Feed Ingredient) contained (g/kg of DM): OM (910), CP (170), crude fiber (46), and ether extract (557). Fatty acid composition (% FFA): C14:0 (8.5), C16:0 (23.2), C20:3 n-6 (1.6), C20:5 n-3 (1.9), C22:5 n-6 (17.7), and C22:6 n-3 (42.3).

Analysis of FAME in hexane was performed on a gas chromatograph (Agilent 6890 N Network System, Palo Alto, CA) with auto injector and fitted with a flame ionization detector. The FAME profile was determined by split injection (1:100) onto a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm i.d., 0.20 µm film thickness; Varian, Middelburg, the Netherlands) using the same programmed temperature gradient method as described in Hervás et al. (2008). Separation of CLA methyl esters was carried out using an HPLC (Shimadzu, model SPE-MA10AVP, Kyoto, Japan) equipped with a diode array detector operated at 233 nm. Three ChromSpher 5 Lipid analytical silver-impregnated columns (250 mm × 4.6 mm i.d. stainless steel; 5 µm particle size; Varian) were used in series. The mobile phase was 0.1% acetonitrile and 0.5% diethyl ether in hexane and was operated isocratically at a flow-rate of 1.0 mL/min. Quantification of individual FAME was made by reference to a milk fat with a known composition (CRM 164; European Community Bureau of Reference, Brussels, Belgium). Individual CLA isomers were identified by comparison with standard mixtures distributed by Nu-Chek Prep Inc. (Elysian, MN). GLC-461 from Nu-Chek Prep Inc. was also used to identify other FA.

Calculations and Statistical Analyses

Milk fat melting point was calculated as described by Jensen and Patton (2000) using FA with a molar

fraction >0.1% in milk fat (97% of total FA). For each individual FA, melting point was obtained from Gunstone et al. (1994). Transfer efficiency of C22:6 n-3 (docosahexaenoic acid; **DHA**) from diet to milk was calculated as [(g of milk fat yield × % DHA in milk fat)/(DMI × % DHA in the TMR)] × 100. Desaturase index was calculated as product of Δ⁹-desaturase/(product of Δ⁹-desaturase + substrate of Δ⁹-desaturase).

All analyses were performed using the SAS software package (version 9.1, SAS Institute Inc., Cary, NC). Data on DMI, and milk yield and composition, as well as FA composition, were analyzed by repeated measurement analysis using the MIXED procedure of SAS and assuming a covariance structure on the basis of Schwarz's Bayesian information model fit criteria. The statistical model included the fixed effects of diet, time, their interaction, and the initial record measured at d 0 (covariate). For all data collected either individually (milk yield and composition) or per lot (DMI and FA composition), the lot was nested within the diet to contrast the effect of the lipid supplementation. Least squares means (adjusted for the covariance) are reported throughout. They were generated and separated using the PDIF option of SAS incorporating the Tukey test for pairwise comparison of treatment means, both for main or interactive effects when they were significant. Pearson correlation coefficients (r) were generated for associations between some FA, milk yield, and fat content with the CORR procedure of SAS using the

Table 2. DMI, milk yield, and milk composition in ewes fed the experimental diets

Item	Treatment ¹					SED ²	P-value ³		
	Control	SO	SOMA ₁	SOMA ₂	SOMA ₃		D	T	D × T
DMI, g/d	3,280	3,585	3,608	3,436	3,459	211.7	NS	**	NS
Yield, g/d									
Milk	2,059	2,153	2,010	2,110	1,928	134.0	NS	***	NS
Protein	107.1	110.1	98.7	103.4	95.5	5.62	NS	***	NS
Fat	125.9 ^{ab}	140.9 ^a	115.7 ^{bc}	118.4 ^{bc}	101.7 ^c	6.80	*	***	**
TS	349.1	372.5	328.7	340.3	306.0	17.65	†	***	†
Composition, g/100 g of raw milk									
Protein	5.22 ^a	5.16 ^a	4.93 ^b	4.95 ^b	4.96 ^b	0.081	*	NS	NS
Fat	6.15 ^{ab}	6.51 ^a	5.75 ^{bc}	5.67 ^{bc}	5.29 ^c	0.212	**	***	***
TS	16.98 ^a	17.35 ^a	16.33 ^b	16.22 ^b	15.88 ^b	0.251	**	***	***

^{a-c}Means within a row with different superscripts differ significantly.

¹Diets included a TMR without lipid supplementation (control) or supplementation with 25 g of sunflower oil/kg of DM plus 0 (SO), 8 (SOMA₁), 16 (SOMA₂), or 24 (SOMA₃) g of marine algae (56.7% ether extract)/kg of DM.

²SED = standard error of the difference.

³Probability of significant effects of experimental diet (D), time on diet (T), and their interaction (D × T). NS = not significant ($P > 0.10$).

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

lot as the experimental unit. Differences were declared significant at $P < 0.05$, and values of $P < 0.10$ were interpreted as tendencies toward significance.

RESULTS

Ewe Performance and Milk Composition

Neither DMI nor milk yield was significantly affected by lipid supplementation ($P > 0.10$; Table 2), and, although there were no changes in milk protein yield ($P = 0.166$), its content was slightly reduced when feeding MA (-4.7% ; $P = 0.030$). Both milk fat content and yield, however, were significantly affected by the inclusion of lipids in the diet. Thus, all MA supplements decreased milk fat content from d 14 onward (Figure 1), reaching a 30% reduction after 28 d on the highest level of MA inclusion (SOMA₃; $P < 0.001$). Changes in TS content mirrored those reported for milk fat, and all SOMA diets reduced the percentage of milk solids ($P = 0.006$).

Saturated and Monoenoic FA

As shown in Table 3, percentages (g/100 g of total FA) of most short- and medium-chain saturates were significantly reduced in supplemented diets, except for C4:0 (slightly increased; $P = 0.009$) and C14:0 (unmodified, $P = 0.149$). Feeding SO significantly decreased C16:0 concentration, but only when fed alone or combined with the lowest dose of MA (SOMA₁). As observed in Figure 2, stearic acid content was increased with the SO (+35%), whereas MA induced a decrease ($P < 0.001$). After only 7 d on SOMA diets, C18:0 aver-

age levels were as low as 2.27% of total FA in SOMA₁ (-66% ; $P < 0.001$) and 1.38% of total FA in SOMA₂ and SOMA₃ (-79% ; $P < 0.001$). Percentages of most odd- and branched-chain FA also diminished with lipid supplementation ($P < 0.05$; Table 3).

There were also important changes in milk content of almost all monounsaturated FA, with significant diet × time interactions. Oleic acid (*cis*-9 C18:1) level was significantly increased with the SO diet (+20%), whereas inclusion of MA diminished it (-29% ; Figure 2). All lipid supplements resulted in greater *trans*-C18:1 percentages, mainly *trans*-10 and *trans*-11, which were remarkably enhanced in SOMA diets ($P < 0.001$). The content of VA increased by 8.6 times in SOMA₃ treatment (averaging 8% of total FA; Figure 2), with this elevated level being sustained throughout the whole monitoring period. Milk content of *trans*-10 C18:1 re-

Figure 1. Temporal changes in milk fat content in ewes fed a TMR without lipid supplementation (control) or supplemented with 25 g of sunflower oil/kg of DM plus 0, 8, 16, or 24 g of marine algae/kg of DM (SO, SOMA₁, SOMA₂, and SOMA₃ diets, respectively). Values are the mean from 2 lots of 5 animals per lot; vertical bars represent the standard error of the means.

mained statistically unmodified when SO was fed alone, but inclusion of MA induced large increases, especially on d 14 of supplementation ($P < 0.001$).

Finally, the milk fat concentration of other *trans*-C18:1 isomers (*trans*-6+7+8, *trans*-9, and *trans*-12) was significantly increased with SO and SOMA₁ treatments (Table 3), except for *trans*-9, which was also augmented with SOMA₂ and SOMA₃ ($P < 0.001$).

Conjugated and Nonconjugated Linoleic Acid

The addition of MA to SO-containing diets resulted in large increases in milk fat RA, much higher than those observed when SO was supplemented alone ($P < 0.001$; Table 3), with the highest concentration corresponding to SOMA₃ treatment. As shown in Figure 2, RA content increased sharply during the first 7 d on SOMA treatments and, after a transient declination, the highest levels were achieved in SOMA₁ and SOMA₃ on d 28 ($P < 0.001$). Addition of SO alone induced a constant increase of RA content over the whole period. *Trans*-7,*cis*-9 CLA doubled its level with the SO and SOMA₁ diets ($P < 0.001$; Table 3). A 9-fold increase in *trans*-9,*cis*-11 CLA ($P < 0.001$; Table 3) was reached after 28 d on SOMA₂ and SOMA₃ treatments (Figure 2). Other CLA isomers, such as *trans*-10,*cis*-12, were present only in very low concentrations (Table 3; Figure 2).

The most abundant nonconjugated PUFA was linoleic acid (*cis*-9,*cis*-12 C18:2), but its level was little modified except for the slight decrease observed with MA ($P = 0.006$). This marine additive significantly augmented the concentration of other, less abundant nonconjugated dienes, such as *trans*-11,*cis*-15 C18:2.

Other Polyunsaturated FA

Lipid inclusion significantly decreased α -linolenic acid (C18:3 n-3) in milk fat, whereas C22:6 n-3 (DHA) content showed a sharp increase after the start of MA supplementation ($P < 0.001$), with relatively stable concentration afterward. However, calculated DHA transfer efficiencies from SOMA diets to milk were very low (0.040–0.058). Increases in other long-chain n-3 PUFA, such as C22:5 (docosapentaenoic acid) or C20:5 (eicosapentaenoic acid), were observed only with the medium and high levels of MA inclusion ($P < 0.001$) and, in any event, their content was always below 0.2% of total FAME. Changes in milk content of n-6 PUFA were of lower magnitude with the exception of the higher levels of C22:5 n-6 obtained with SOMA diets (Table 3).

DISCUSSION

Ewe Performance and Milk Composition

Inclusion of free vegetable oils in dairy ewe diet has been reported to have no apparent detrimental effect on DMI and milk yield (Pulina et al., 2006; Gómez-Cortés et al., 2008a; Hervás et al., 2008), and although decreases in feed consumption are frequently observed when feeding marine algae to sheep or cows, this reduction does not always affect milk yield (Franklin et al., 1999; Offer et al. 2001; Papadopoulos et al., 2002).

In relation to milk composition, the 6% reduction of the TS content found with SOMA treatments was the consequence of the significant decrease in milk protein and fat contents caused by the inclusion of these lipids in the diet. Reductions in milk protein are frequently observed when the diet of dairy ewes is supplemented with oil (Pulina et al., 2006; Gómez-Cortés et al., 2008b) and are generally attributed to reduced AA availability. Previous studies have shown no evidence of MFD in dairy ewes fed free vegetable oils (Pulina et al., 2006; Gómez-Cortés et al., 2008a,b; Hervás et al., 2008), but few experiments have examined the effect of unprotected marine lipids in this species. Furthermore, the reported effects of the latter on ewe milk fat content are very inconsistent, with reductions, no changes, and even increases (Papadopoulos et al., 2002; Reynolds et al., 2006; Capper et al., 2007), which could be mostly accounted for by differences in basal diet composition and lipid dosage.

Milk Fat Depression and Putative Inhibitors

In dairy cows, MFD induced by the use of free vegetable oils and high-concentrate diets is characterized by increased milk fat concentration of *trans*-10,*cis*-12 CLA, a biohydrogenation (BH) intermediate with antilipogenic effect not only in this ruminant species (Chilliard et al., 2007; Shingfield et al., 2009) but also in sheep (Lock et al., 2006). However, the very low levels and the lack of variation in this isomer (Table 3; Figure 2) would suggest that other inhibitors of milk fat synthesis should also be involved. Furthermore, greater increases in *trans*-10,*cis*-12 CLA in the absence of MFD have previously been reported in dairy sheep fed either low- or high-concentrate diets (Reynolds et al., 2006; Gómez-Cortés et al., 2008a; Hervás et al., 2008).

Postprandial infusion experiments have provided tentative evidence that *cis*-10,*trans*-12 and *trans*-9,*cis*-11 CLA also exert antilipogenic effects (Perfield et al., 2007; Harvatine et al., 2009). The former FA was not detected in the present study, but the remarkable in-

Table 3. Fatty acid (FA) profile of the milk from ewes fed the experimental diets

Fatty acid, g/100 g of total FA methyl esters	Treatment ¹						P-value ³		
	Control	SO	SOMA ₁	SOMA ₂	SOMA ₃	SED ²	D	T	D × T
Saturated FA									
4:0	3.71 ^c	4.00 ^a	4.06 ^a	4.00 ^{ab}	3.84 ^{bc}	0.096	**	NS	*
6:0	3.49 ^a	3.34 ^b	3.02 ^d	3.29 ^{bc}	3.22 ^c	0.040	***	***	*
8:0	3.47 ^a	3.08 ^b	2.77 ^c	3.02 ^b	2.92 ^{bc}	0.085	**	***	***
10:0	10.90 ^a	8.80 ^b	8.38 ^b	8.73 ^b	8.27 ^b	0.390	*	***	***
12:0	5.99 ^a	4.70 ^b	4.83 ^b	4.61 ^b	4.40 ^b	0.279	*	***	***
13:0 <i>anteiso</i>	0.07 ^a	0.04 ^b	0.05 ^b	0.04 ^b	0.05 ^b	0.005	**	NS	*
13:0	0.21 ^a	0.13 ^b	0.14 ^b	0.12 ^b	0.13 ^b	0.019	*	***	***
14:0 <i>iso</i>	0.11 ^a	0.10 ^{ab}	0.09 ^{bc}	0.08 ^c	0.08 ^c	0.006	**	***	***
14:0	11.79	10.80	11.76	11.40	11.11	0.385	NS	***	*
15:0 <i>iso</i>	0.20 ^a	0.18 ^b	0.18 ^b	0.16 ^c	0.18 ^b	0.007	*	**	NS
15:0 <i>anteiso</i>	0.43 ^a	0.39 ^{ab}	0.36 ^{bc}	0.32 ^c	0.33 ^c	0.020	*	***	*
15:0	0.95 ^a	0.79 ^b	0.80 ^b	0.74 ^c	0.73 ^c	0.010	***	***	***
16:0 <i>iso</i>	0.40 ^a	0.29 ^b	0.30 ^b	0.23 ^d	0.25 ^c	0.020	***	***	NS
16:0	25.94 ^a	22.44 ^c	24.29 ^b	24.84 ^{ab}	25.91 ^a	0.578	**	***	**
17:0	0.53 ^a	0.43 ^b	0.44 ^b	0.42 ^b	0.41 ^b	0.025	*	***	**
18:0	6.57 ^b	8.84 ^a	3.16 ^c	2.37 ^d	2.33 ^d	0.207	***	***	***
18:0 <i>iso</i>	0.06	0.06	0.06	0.06	0.06	0.006	NS	**	NS
20:0	0.17 ^a	0.17 ^a	0.15 ^b	0.14 ^b	0.14 ^b	0.007	*	*	*
21:0	0.07 ^a	0.06 ^b	0.05 ^b	0.05 ^b	0.05 ^b	0.004	*	***	†
22:0	0.13 ^b	0.15 ^a	0.17 ^a	0.17 ^a	0.17 ^a	0.008	*	***	*
23:0	0.06 ^b	0.05 ^b	0.09 ^a	0.09 ^a	0.09 ^a	0.005	**	***	***
24:0	0.04 ^c	0.04 ^c	0.06 ^b	0.07 ^a	0.07 ^a	0.004	***	***	**
Monounsaturated FA									
10:1 + 12:0 <i>iso</i>	0.49 ^a	0.38 ^b	0.34 ^b	0.31 ^b	0.32 ^b	0.034	*	***	***
<i>cis</i> -9 14:1	0.23 ^a	0.20 ^{bc}	0.23 ^{ab}	0.19 ^c	0.18 ^c	0.013	*	*	†
15:1	0.14 ^a	0.11 ^b	0.13 ^{ab}	0.13 ^{ab}	0.15 ^a	0.010	†	**	**
<i>trans</i> -8 16:1	0.06 ^b	0.09 ^b	0.22 ^a	0.21 ^a	0.18 ^a	0.019	**	***	*
<i>trans</i> -9 16:1 + 17:0 <i>iso</i>	0.39 ^d	0.49 ^c	0.80 ^b	0.75 ^b	0.88 ^a	0.023	***	***	***
<i>cis</i> -7 16:1	0.29	0.30	0.28	0.27	0.27	0.016	NS	*	†
<i>cis</i> -9 16:1 + 17:0 <i>anteiso</i>	1.41 ^a	1.15 ^c	1.22 ^b	1.05 ^d	1.04 ^d	0.030	***	***	*
<i>cis</i> -13 16:1	0.17 ^a	0.09 ^b	0.08 ^{bc}	0.07 ^c	0.08 ^{bc}	0.008	***	NS	**
17:1	0.20 ^a	0.16 ^b	0.15 ^{bc}	0.13 ^d	0.13 ^{cd}	0.008	**	***	**
<i>trans</i> -6+7+8 18:1	0.19 ^b	0.41 ^a	0.43 ^a	0.20 ^b	0.17 ^b	0.020	***	***	***
<i>trans</i> -9 18:1	0.18 ^c	0.38 ^a	0.42 ^a	0.26 ^b	0.26 ^b	0.017	***	***	***
<i>trans</i> -10 18:1	0.34 ^c	0.65 ^c	4.04 ^b	4.99 ^a	3.62 ^b	0.239	***	***	***
<i>trans</i> -11 18:1 (vaccenic acid)	0.93 ^d	2.80 ^c	6.91 ^b	7.25 ^b	7.98 ^a	0.169	***	***	***
<i>trans</i> -12 18:1	0.26 ^d	0.47 ^b	0.57 ^a	0.34 ^c	0.34 ^c	0.025	***	***	***
<i>trans</i> -15 + <i>cis</i> -11 18:1	0.32 ^b	0.37 ^b	0.48 ^a	0.45 ^a	0.43 ^a	0.021	**	***	***
<i>trans</i> -16 + <i>cis</i> -14 18:1	0.33 ^b	0.48 ^a	0.33 ^b	0.22 ^c	0.22 ^c	0.015	***	***	***
<i>cis</i> -9 18:1	13.32 ^b	16.01 ^a	9.84 ^c	9.34 ^c	9.19 ^c	0.283	***	***	***
<i>cis</i> -12 18:1	0.34 ^b	0.70 ^a	0.19 ^c	0.13 ^{cd}	0.12 ^d	0.025	***	*	***
<i>cis</i> -13 18:1	0.05 ^c	0.06 ^b	0.07 ^a	0.08 ^a	0.07 ^{ab}	0.004	**	**	*
<i>cis</i> -15 18:1	0.08 ^c	0.11 ^a	0.10 ^{ab}	0.09 ^{bc}	0.09 ^{bc}	0.009	*	*	*
<i>cis</i> -11 20:1	0.07 ^b	0.07 ^b	0.10 ^a	0.11 ^a	0.10 ^a	0.003	***	***	*
<i>cis</i> -13 22:1	0.02 ^d	0.02 ^d	0.03 ^c	0.05 ^b	0.06 ^a	0.002	***	***	***
24:1	0.02	0.03	0.02	0.03	0.03	0.004	NS	**	NS
Nonconjugated C18:2									
<i>trans</i> -9, <i>cis</i> -12	0.03 ^c	0.04 ^c	0.10 ^{ab}	0.11 ^a	0.09 ^b	0.005	***	***	***
<i>trans</i> -11, <i>cis</i> -15	0.05 ^c	0.04 ^c	0.21 ^b	0.28 ^a	0.30 ^a	0.019	***	***	***
<i>cis</i> -9, <i>cis</i> -12	2.37 ^a	2.23 ^{ab}	2.03 ^c	2.04 ^{bc}	1.86 ^c	0.075	**	***	***
<i>cis</i> -9, <i>cis</i> -15	0.08 ^a	0.06 ^b	0.05 ^c	0.05 ^c	0.05 ^{bc}	0.005	***	**	*
Other 18:2 ⁴	0.20 ^c	0.28 ^a	0.24 ^b	0.16 ^d	0.15 ^d	0.010	***	*	*
Other 18:2 ⁵ + <i>cis</i> -16 18:1	0.19 ^{bc}	0.27 ^a	0.23 ^b	0.18 ^c	0.17 ^c	0.013	**	***	***
Conjugated C18:2									
<i>cis</i> -9, <i>trans</i> -11 (rumenic acid)	0.44 ^d	1.23 ^c	2.78 ^b	2.58 ^b	3.22 ^a	0.114	***	***	***
<i>trans</i> -7, <i>cis</i> -9	0.04 ^d	0.08 ^b	0.09 ^a	0.05 ^c	0.04 ^{cd}	0.002	***	***	***
<i>trans</i> -8, <i>cis</i> -10	0.02 ^c	0.03 ^b	0.04 ^a	0.03 ^{ab}	0.03 ^a	0.003	***	***	***
<i>trans</i> -9, <i>cis</i> -11	0.02 ^d	0.03 ^c	0.10 ^b	0.11 ^a	0.10 ^b	0.005	***	***	***
<i>trans</i> -10, <i>cis</i> -12	<0.01 ^c	<0.01 ^{bc}	0.01 ^{ab}	0.01 ^a	0.01 ^{abc}	0.002	*	***	NS
<i>trans</i> -11, <i>cis</i> -13	0.01 ^b	0.01 ^b	0.02 ^a	0.02 ^a	0.02 ^a	0.002	**	***	NS
<i>trans</i> -12, <i>trans</i> -14	0.01	0.01	0.01	0.01	0.01	0.001	NS	***	NS
<i>trans</i> -11, <i>trans</i> -13	0.03 ^a	0.03 ^a	0.02 ^b	0.02 ^b	0.02 ^b	0.002	**	***	*
Other <i>trans-trans</i>	0.01 ^c	0.02 ^b	0.03 ^a	0.03 ^a	0.03 ^{ab}	0.002	***	***	*

Continued

Table 3 (Continued). Fatty acid (FA) profile of the milk from ewes fed the experimental diets

Fatty acid, g/100 g of total FA methyl esters	Treatment ¹					SED ²	P-value ³		
	Control	SO	SOMA ₁	SOMA ₂	SOMA ₃		D	T	D × T
Other polyunsaturated FA									
16:2 n-6	0.02 ^b	0.02 ^b	0.05 ^a	0.06 ^a	0.06 ^a	0.005	***	***	*
18:3 n-6	0.08 ^a	0.06 ^b	0.06 ^b	0.05 ^b	0.05 ^b	0.004	***	***	*
18:3 n-3	0.53 ^a	0.41 ^b	0.37 ^c	0.36 ^c	0.34 ^d	0.013	***	†	NS
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.03	0.03	0.03	0.03	0.03	0.003	NS	**	NS
18:4 n-3	0.01 ^c	0.02 ^b	0.02 ^b	0.02 ^{ab}	0.03 ^a	0.002	*	***	NS
20:2 n-6	0.02	0.02	0.02	0.03	0.03	0.003	NS	†	NS
20:3 n-3	0.02 ^{bc}	0.01 ^c	0.02 ^{ab}	0.02 ^{ab}	0.03 ^a	0.003	*	*	†
20:4 n-6	0.16 ^c	0.12 ^d	0.17 ^{bc}	0.20 ^b	0.24 ^a	0.014	**	***	***
20:5 n-3 (eicosapentaenoic acid)	0.05 ^c	0.04 ^c	0.05 ^c	0.09 ^b	0.10 ^a	0.004	***	***	***
22:4 n-6	0.03 ^d	0.03 ^d	0.04 ^c	0.05 ^b	0.05 ^a	0.001	***	***	**
22:5 n-6	0.01 ^d	0.02 ^d	0.11 ^c	0.28 ^b	0.33 ^a	0.014	***	***	***
22:5 n-3 (docosapentaenoic acid)	0.10 ^e	0.07 ^d	0.10 ^c	0.13 ^b	0.15 ^a	0.004	***	***	***
22:6 n-3 (docosahexaenoic acid)	0.03 ^d	0.02 ^d	0.17 ^c	0.46 ^b	0.57 ^a	0.021	***	***	***

^{a-d}Means within a row with different superscripts differ significantly.

¹Diets included a TMR without lipid supplementation (control) or supplementation with 25 g of sunflower oil/kg of DM plus 0 (SO), 8 (SOMA₁), 16 (SOMA₂), or 24 (SOMA₃) g of marine algae (56.7% ether extract)/kg of DM.

²SED = standard error of the difference.

³Probability of significant effects of experimental diet (D), time on diet (T), and their interaction (D × T). NS = not significant ($P > 0.10$).

⁴*trans*-9,*trans*-12 + *cis*-9,*trans*-13 + *trans*-8,*cis*-12 C18:2.

⁵*trans*-8,*cis*-13 + *cis*-9,*trans*-12 C18:2.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

creases in *trans*-9,*cis*-11 C18:2 could partially explain the MFD arising in ewes consuming SOMA diets. Previous evidence in cows (Roy et al., 2006; Perfield et al., 2007) indicates that this CLA isomer inhibits milk fat synthesis, but with a much lower efficacy than *trans*-10,*cis*-12 C18:2.

The inverse relationship between milk fat yield and *trans*-10 C18:1 ($r = -0.71$; $P < 0.001$) might suggest that this FA is another candidate for the inhibition of milk fat synthesis. However, a 4-d abomasal infusion of 40 g/d of this *trans*-C18:1 isomer was not effective in reducing the milk fat percentage in cows (Lock et al., 2007), which may be the result of the low enrichment of *trans*-10 C18:1 during infusions (Kadegowda et al., 2008). A more recent study (Kadegowda et al., 2009) supports the idea that *trans*-10 C18:1 reaching the mammary gland could potentially induce MFD by decreasing lipogenic gene expression. Furthermore, infusions of approximately 90 g/d of *trans*-10 C18:1 for a 5-d period reduced milk fat secretion, although the authors (Shingfield et al., 2009) suggest that this *trans*-FA was about 40- to 50-fold less effective than *trans*-10,*cis*-12 C18:2. Interestingly, in that study, the level of milk *trans*-10 C18:1 was about 4%, similar to that observed here for SOMA diets (Table 3). However, it is noteworthy that higher levels (about 6%) have previously been found in dairy ewes supplemented with vegetable oils without concomitant reductions in milk fat (Gómez-Cortés et al., 2008a; Hervás et al., 2008), which would rule out *trans*-10 C18:1 as the principal

factor responsible for the reduction observed in milk fat content.

The high correlation between *trans*-10 C18:1 and *trans*-9,*cis*-11 C18:2 ($r = 0.93$; $P < 0.001$) might support the hypothesis that they have a common precursor or are produced by a common rumen bacteria (Roy et al., 2006; Shingfield et al., 2006; Gama et al., 2008), and although the low concentration of *trans*-9,*cis*-11 CLA makes it unlikely that it may be the principal component responsible for MFD (Perfield et al., 2007), *trans*-9,*cis*-11 CLA and milk fat content were also negatively correlated ($r = -0.78$; $P < 0.001$). Accordingly, a joint action of *trans*-9,*cis*-11 C18:2 and *trans*-10 C18:1, together with that of other unidentified intermediates, would seem to be a feasible explanation for MFD in SOMA treatments (Perfield et al., 2007; Kadegowda et al., 2008, 2009; Shingfield et al., 2009), but mechanisms other than direct inhibition may also be involved. In this regard, several authors have speculated about an effect of the maintenance of milk fat fluidity on milk fat secretion (Shingfield et al., 2006; Chilliard et al., 2007; Gama et al., 2008) related to the incorporation of oleic acid and short-chain FA (C4:0–C10:0) into triglycerides as the principal means of ensuring milk fat liquidity at body temperature (Timmen and Patton, 1988). Thus, the known inhibition of marine lipids on C18:1 ruminal saturation (Or-Rashid et al., 2008), reducing therefore the availability of C18:0 for endogenous mammary synthesis of oleic acid, plus the contribution of decreased de novo FA synthesis, would detrimentally affect the

Figure 2. Temporal changes in C18:0, *cis*-9 C18:1, *trans*-11 C18:1, *cis*-9,*trans*-11 CLA, *trans*-10 C18:1, *trans*-10,*cis*-12 CLA, and *trans*-9,*cis*-11 CLA (g/100 g of fatty acids methyl esters) in ewes fed a TMR without lipid supplementation (control) or supplemented with 25 g of sunflower oil/kg of DM plus 0, 8, 16, or 24 g of marine algae/kg of DM (SO, SOMA₁, SOMA₂, and SOMA₃ diets, respectively). Values are the mean from 2 lots of 5 animals per lot; vertical bars represent the standard error of the means. CLA = conjugated linoleic acid; RA = rumenic acid.

Table 4. Fatty acid (FA) composition, indices of desaturase activity, n-6:n-3 ratio, and melting point of the milk from ewes fed the experimental diets

Fatty acid composition, g/100 g of FA methyl esters	Treatment ¹					SED ²	P-value ³		
	Control	SO	SOMA ₁	SOMA ₂	SOMA ₃		D	T	D × T
According to degree of saturation									
Saturated FA	75.40 ^a	69.00 ^b	65.07 ^c	65.01 ^c	64.91 ^c	0.383	***	***	***
Monounsaturated FA	19.73 ^d	25.48 ^c	26.98 ^a	26.54 ^{ab}	25.90 ^{bc}	0.312	***	***	***
Polyunsaturated FA	4.50 ^d	5.47 ^c	7.82 ^b	8.03 ^b	8.91 ^a	0.120	***	***	***
According to origin ⁴									
<C16	42.60 ^a	37.18 ^b	37.07 ^b	37.25 ^b	36.14 ^b	0.977	**	***	***
C16	28.65 ^{ab}	24.92 ^c	27.31 ^b	27.51 ^{ab}	28.79 ^a	0.524	**	***	***
>C16	29.00 ^c	37.80 ^a	35.61 ^b	35.24 ^b	35.08 ^b	0.581	***	***	***
Desaturase index									
<i>cis</i> -9 C14:1/(C14:0 + <i>cis</i> -9 C14:1)	0.019 ^a	0.019 ^a	0.019 ^a	0.017 ^b	0.015 ^b	0.0009	**	***	†
<i>cis</i> -9 C16:1/(C16:0 + <i>cis</i> -9 C16:1)	0.051 ^a	0.049 ^{ab}	0.046 ^{bc}	0.044 ^c	0.040 ^d	0.0114	**	***	*
<i>cis</i> -9 C18:1/(C18:0 + <i>cis</i> -9 C18:1)	0.670 ^c	0.645 ^d	0.768 ^b	0.817 ^a	0.820 ^a	0.0043	***	***	***
<i>cis</i> -9, <i>trans</i> -11 C18:2/(<i>trans</i> -11 C18:1 + <i>cis</i> -9, <i>trans</i> -11 C18:2)	0.321 ^a	0.287 ^b	0.264 ^c	0.267 ^c	0.327 ^a	0.0073	***	***	***
n-6:n-3 ratio	3.47 ^b	4.21 ^a	3.27 ^b	2.42 ^c	2.12 ^c	0.119	***	***	***
Milk melting point, °C	34.6 ^b	34.0 ^c	35.1 ^a	34.5 ^b	34.6 ^b	0.14	***	***	*

^{a-d}Means within a row with different superscripts differ significantly.

¹Diets included a TMR without lipid supplementation (control) or supplementation with 25 g of sunflower oil/kg of DM plus 0 (SO), 8 (SOMA₁), 16 (SOMA₂), or 24 (SOMA₃) g of marine algae (56.7% ether extract)/kg of DM.

²SED = standard error of the difference.

³Probability of significant effects of experimental diet (D), time on diet (T), and their interaction (D × T). NS = not significant ($P > 0.10$).

⁴<C16 represent de novo synthesized FA, >C16 represent preformed FA taken up from circulation, and C16 FA are derived from both sources.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 3. Temporal changes in <C16 and >C16 fatty acid yield (g/d) in ewes fed a TMR without lipid supplementation (control) or supplemented with 25 g of sunflower oil (SO)/kg of DM plus 0, 8, 16, or 24 g of marine algae (MA)/kg of DM (SO, SOMA₁, SOMA₂, and SOMA₃ diets, respectively). Values are the mean from 2 lots of 5 animals per lot; vertical bars represent the standard error of the means.

maintenance of milk fat liquidity at body temperature. Moreover, the augmentation in *trans*-FA (mainly C18:1 isomers), with higher melting points than their equivalent *cis*-isomers (Gunstone et al., 1994), might also contribute to reduce milk fat fluidity because milk fat globules with melting points higher than body temperature could not be secreted (Timmen and Patton, 1988; Gama et al., 2008). Major changes in milk FA composition induced numerically minor variations in calculated milk fat melting point (between -0.7 and $+0.7^{\circ}\text{C}$; Table 4), even lower than those reported in the milk of cows fed fish oil (Gama et al., 2008), which

gives an idea of the high regulatory capacity of the mammary gland.

Furthermore, the activity of the Δ^9 -desaturase in the mammary gland of ruminants is also thought to occur as a mechanism to maintain and regulate the fluidity of milk fat for efficient secretion. Although significant differences were observed for all the calculated desaturase indices (Table 4), only changes in *cis*-9 C18:1/(C18:0 + *cis*-9 C18:1) were of quantitative importance. According to Gama et al. (2008), this could be attributable to either an increase in the specific activity of Δ^9 -desaturase for stearic acid when feeding marine lipids or a higher uptake of oleic acid coming from adipose tissues. Both responses may result from a physiological adaptation to the increased supply of *trans*-C18:1 FA and the decline in the stearic acid uptake and availability for mammary oleic acid synthesis.

In addition, as previously reported for CLA-induced MFD (Lock et al., 2006; Harvatine et al., 2009), lipid supplements reduced the milk content of FA with fewer than 16 C (i.e., synthesized de novo in the mammary gland) and increased those with more than 16 C (i.e., originated from mammary uptake of circulating FA), suggesting further mechanisms by which the mammary gland can adapt to altered supply of FA. To give a clearer insight into changes in mammary fat secretion, data of de novo and preformed FA yields are shown in Figure 3. Thus, when SO was fed alone, reduced de novo FA yield (from d 14 onward; $P < 0.05$) was alleviated by an increase in the secretion of >C16 FA ($P < 0.01$). However, the greater decrease in <C16 FA yield induced by the addition of MA ($P < 0.05$) was not compensated for by the transient increase in long-chain FA yield.

Saturated and Monoenoic FA

It is now well established that increases in the supply of long-chain FA to the mammary gland alter the synthesis of short- and medium-chain saturates (Pulina et al., 2006; Chilliard et al., 2007; Kadegowda et al., 2009). Because high intakes of saturated FA could be related to an increased risk of cardiovascular disease and development of the metabolic syndrome (Shingfield et al., 2008), the effect of SO and MA supplementation (Table 4) would have a positive effect on the nutritional value of milk fat. It is, however, noteworthy that the specific role of saturated FA should be considered individually in relation to their putative effect on human health (see review by Shingfield et al., 2008).

The decrease in C18:0 and *cis*-9 C18:1 together with the increase in *trans*-C18:1 in SOMA diets milk fat would reflect the inhibitory action of the long-chain n-3 PUFA present in MA, mainly DHA (AbuGhazaleh

and Jenkins, 2004), on *trans*-C18:1 ruminal reduction (Loor et al., 2005; Sinclair et al., 2005; Or-Rashid et al., 2008). As a consequence, SOMA treatments resulted in the greatest percentage of *trans*-C18:1, mainly VA and *trans*-10, 2 intermediaries of linoleic acid BH pathways (Jenkins et al., 2008). Other minor *trans*-C18:1 (*trans*-6+7+8, *trans*-9, and *trans*-12) could also derive from isomerization of oleic acid (26% of total FA in sunflower oil) because BH of this latter FA involves the formation of several isomers of *trans*-monoenes further to *trans*-10 and *trans*-11 C18:1 (Jenkins et al., 2008).

High contents of VA in bovine milk are better maintained with high levels of forage, or low lipid supplementation, or both (Palmquist et al., 2005; Chilliard et al., 2007; Cruz-Hernández et al., 2007), whereas addition of marine lipids or linoleic oil- and starch-rich diets have been related to a shift in ruminal BH toward increased formation of *trans*-10 C18:1 at the expense of VA (Roy et al., 2006; Chilliard et al., 2007; Or-Rashid et al., 2008). It is remarkable that high levels of *trans*-10 C18:1 in ovine milk appear to be accompanied by similar or even higher contents of VA (Reynolds et al., 2006), whereas in dairy cows, there is a pronounced inverse relationship between these 2 FA, so the *trans*-10 C18:1 content may amply exceed that of VA (Roy et al., 2006; Shingfield et al., 2006; Boeckaert et al., 2008). This highlights the importance of interspecific differences among dairy ruminants, as has been stated in the case of goats and cows (Chilliard et al., 2007). Even though notable and greater *trans*-10 C18:1 levels were previously achieved in dairy ewes with the inclusion of linoleic-rich oils in high-starch diets (Gómez-Cortés et al., 2008a; Hervás et al., 2008), an adequate fiber content probably prevented altered ruminal biohydrogenation toward *trans*-10 C18:1 ruminal formation in the SO treatment but not in MA-containing diets (Roy et al., 2006; Shingfield et al., 2006), where rumen environment might be altered by MA. This hypothesis would be in line with the decrease of branched- and odd-chain FA contents observed mainly in MA treatments. Unexpectedly, the high *trans*-10 C18:1 concentration observed in SOMA treatments decreased significantly in SOMA₁ on d 28, which might indicate adaptation of the rumen microbiota to MA consumption. This speculation could be supported by the concomitant variations in *trans*-9, *cis*-11 CLA (decrease) and VA and RA (increases) and the slight recovery in milk fat content.

Conjugated and Nonconjugated Linoleic Acid

Because of the transient nature of RA formed in the rumen, endogenous synthesis from VA through mammary Δ^9 -desaturase is considered the principal origin

of this CLA isomer in ruminant milk (Palmquist et al., 2005; Pulina et al., 2006; Shingfield et al., 2008). Thus, those diets inducing the largest accumulation of VA resulted also in the highest milk RA concentrations ($r = 0.97$; $P < 0.001$). Temporal variations in RA (Figure 1) contrast with the situation in dairy cows, where a progressive, time-dependent reduction in this FA is frequently found after initial increases following lipid supplementation of starch-rich diets (Shingfield et al., 2006; Chilliard et al., 2007).

Changes in nonconjugated C18:2 were less remarkable, and although mean intake of linoleic acid increased by 53 g/d with lipid supplementation, its concentration in milk fat was unmodified with SO and even decreased with SOMA treatments, probably because the usually high ruminal BH of linoleic acid can be further increased with marine lipid supplements (up to 90% according to Sinclair et al., 2005). The notable rise in milk *trans*-C18:1 would also support this hypothesis.

Regarding other nonconjugated C18:2 FA detected in milk fat, *trans*-11, *cis*-15 C18:2 was the most extensively modified by lipid supplementation, reaching on average a 6-fold increase with SOMA₂ and SOMA₃ (0.30% of total FA). Identified as an intermediate of C18:3 n-3 (α -linolenic acid) metabolism (Jenkins et al., 2008), its content is frequently greater in milk fat when marine oils are fed (Shingfield et al., 2006; Chilliard et al., 2007), which suggests a putative inhibitory effect of MA long-chain n-3 PUFA on the complete BH process of α -linolenic acid (Loor et al., 2005).

Other Polyunsaturated FA

Diet supplementation with SO plus MA offered some additional changes pointing toward a healthier milk FA profile, such as the dose-dependent improvement in DHA concentration and the decrease in the n-6:n-3 FA ratio, of great importance in reducing the risk of cardiovascular diseases (Simopoulos, 2008). However, despite the high DHA content of the MA supplement (42% of total FA), these increases appear limited and represented an average transfer efficiency of only 5%, in line with transferences observed by other authors in cows and sheep (Papadopoulos et al., 2002; Loor et al., 2005; Chilliard et al., 2007) but lower than some previous reports in dairy ewes (Reynolds et al., 2006; Capper et al., 2007). The ruminal BH of long-chain n-3 PUFA (Loor et al., 2005; Sinclair et al., 2005; Castañeda-Gutiérrez et al., 2007) may partially account for their low transfer efficiencies into milk. Preferential partitioning of n-3 PUFA into some plasma lipid fractions that are poorly taken up by the mammary gland, such as plasma cholesterol ester and phospholipids (Offer et

al., 1999; Kitessa et al., 2001; Chilliard et al., 2007), would also help to explain these results.

CONCLUSIONS

Supplementation of the diet of ewes with 3 incremental levels of MA and a moderate amount of SO affected animal performance by reducing the milk content of fat and protein, even with the lowest level of MA inclusion. Although all lipid supplements lowered the yield of FA originating from de novo synthesis (<C16), sustained increase in the output of long-chain FA prevented MFD when only SO was added. Marine algae-induced MFD might have been related to the joint action of some putative fat synthesis inhibitors, such as *trans*-9,*cis*-11 CLA and probably *trans*-10 C18:1, as well as to the effect on milk fat fluidity of the decrease in C18:0 and subsequently in *cis*-9 C18:1 content together with the rise in *trans*-FA contents. The combination of SO and MA, especially with the highest level of the latter, proved to be very effective for improving the milk concentration of some bioactive components, such as RA, VA, and DHA, and reducing the n-6:n-3 FA ratio. However, despite changes induced by SOMA supplementation in the milk FA profile that pointed to an enhanced nutritional value of the milk fat, large increases in other *trans*-FA, mainly *trans*-10 C18:1, were also observed.

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