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Comparison of milk fatty acid responses during fish oil- and *trans*-10 *cis*-12 18:2-induced milk fat depression in dairy ewes

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ABSTRACT

The direct comparison between milk fat depression (MFD) caused by the addition of *trans*-10 *cis*-12 18:2 (a conjugated linoleic acid –CLA– isomer) or marine lipids to the diet of dairy ewes may help to elucidate the origin of this syndrome. Therefore, 12 lactating sheep were divided in 3 lots and offered a diet without supplementation (control) or supplemented with 2% DM of fish oil (FO) or 1.1% DM of a rumen-protected product rich in *trans*-10 *cis*-12 18:2 (CLA) for 27 days to compare the responses in terms of animal performance and milk fatty acid (FA) profile. Both supplemented diets (FO and CLA) decreased the milk fat content in a similar manner (–18% compared with the control). On the other hand, responses in milk FA profiles differed significantly and support that marine lipid-induced MFD is not

mediated by the effects of *trans*-10 *cis*-12 18:2. However, a comparison of changes in the molar production of milk FA show that de novo FA synthesis was affected similarly in FO and CLA treatments and more strongly than FA uptake, which implies that both types of MFD might share common mechanisms. The results point to the involvement of less well-known potentially antilipogenic metabolites (such as intermediates of 18:3n-3 biohydrogenation or ruminal hydration and oxidation) in the low-milk fat syndrome in ewes fed FO and seem to downplay the relevance of changes in the milk fat melting point as a major mechanism responsible for FO-induced MFD.

Key words: conjugated linoleic acid, marine oil, lipid, mammary gland, sheep

Abbreviations: ADF, acid detergent fibre; aNDF, neutral detergent fibre; BH, biohydrogenation; BW, body weight; CLA, conjugated linoleic acid; DM, dry matter; FA, fatty acid; FAME, fatty acid methyl ester; FO, fish oil; GC, gas chromatography; MFD, milk fat depression; TMR, total mixed ration.

1. Introduction

The low-fat milk syndrome, commonly referred to as milk fat depression (MFD), has perplexed ruminant nutritionists for over a century and remains an active research area (Bauman et al., 2011). Bauman and Griinari (2001) proposed that diet-induced MFD was due to changes in ruminal lipid metabolism, leading to increased formation of specific biohydrogenation (BH) intermediates that exert antilipogenic effects. This role was initially attributed to *trans*-10 *cis*-12 18:2 because it is the only intermediate that has been unequivocally shown to inhibit milk fat synthesis. However, increases in this conjugated linoleic acid (CLA) isomer cannot explain the fat reductions occurring in marine lipid-induced MFD, which suggests that other intermediates or mechanisms should be involved (Shingfield and Griinari, 2007; Bichi et al., 2013). In line with this, several recent works highlight the need to further investigate the potential antilipogenic action of other fatty acid (FA) intermediates, such as *trans*-10 18:1, *trans*-9 *cis*-11 18:2, *trans*-10 *cis*-15 18:2, or *trans* 20- and 22-carbon FA (e.g., Alves and Bessa, 2014; Kairenius et al., 2015), as well as the contribution of more general mechanisms, such as the maintenance of milk fat fluidity (Shingfield and Griinari, 2007; Toral et al., 2013), to explain this diet-induced MFD.

Furthermore, a different distribution of the molar yield of milk short-, medium- or long-chain FA has been observed in cows with MFD caused by marine lipids or *trans*-10 *cis*-12 18:2 (e.g., Baumgard et al., 2000; Shingfield et al., 2003; Rego et al., 2005), which suggests that the major mechanisms involved in milk fat synthesis (i.e., de novo FA synthesis and uptake of preformed FA from plasma) would not be equally inhibited. In dairy sheep, information regarding marine lipid-induced MFD is still very scant. Nonetheless, the responses in terms of the contribution of de novo synthesis or uptake appear to be similar to those observed when ewes receive *trans*-10 *cis*-12 18:2 (Lock et al., 2006; Sinclair et al.,

2007; Toral et al., 2010a), which contrasts with the expectation based on the results recorded in cows.

In any case, we are not aware of any direct comparison of the effect of *trans*-10 *cis*-12 18:2 and marine lipid addition to the diet. Therefore, this study was conducted in dairy sheep fed diets supplemented with either fish oil or *trans*-10 *cis*-12 18:2 to directly compare the responses in terms of animal performance and, especially, milk FA profile.

2. Material and methods

2.1. Animals, experimental design and management

All experimental procedures were approved and completed in accordance with the Spanish Royal Decree 53/2013 for the protection of animals used for experimental purposes. Twelve lactating Assaf ewes (body weight, BW = 72.5±3.21 kg; 43±2.7 days in milk) were randomly divided into 3 groups (n = 4) balanced for milk production and composition, BW, days in milk and parity. These groups were assigned to 1 of 3 dietary treatments: a total mixed ration (TMR, forage:concentrate ratio 40:60) without lipid supplementation (Control) or supplemented with 2% DM of fish oil (FO treatment) or 1.1% DM of a rumen-protected CLA product (CLA treatment). The ingredients of the experimental diets, which were prepared weekly and included molasses to reduce selection of dietary components, are presented in Table 1.

The ewes were milked daily at approximately 08:30 and 18:00 h in a 1×10 stall-milking parlour (DeLaval, Madrid, Spain) and fed ad libitum after each milking. All animals received the control diet for a 2-week adaptation period, and then, the experiment lasted for 4 more weeks. Clean drinking water was always available.

2.2. Measurements and sampling procedures

Representative samples of the experimental diets and lipid supplements were collected in triplicate and stored at –30ºC until analysis. Feed intake was measured weekly by weighing the amount of DM offered and refused by each lot.

On the last 3 days of the adaptation period, and after 25 and 27 days on treatments, milk yield was recorded and individual milk samples were collected and composited according to morning and evening milk yield. One aliquot was preserved with bronopol (D&F Control Systems, San Ramon, USA) and stored at 4ºC until analysed for fat, protein, lactose and total solids contents. Milk FA composition was determined in untreated samples that were stored at –30ºC until analysis.

2.3. Chemical analysis

Samples of TMR were analysed for DM (ISO 6496:1999), ash (ISO 5984:2002) and crude protein (ISO 5983-2:2009). Neutral and acid detergent fibres (aNDF and ADF) were determined using an Ankom^{2000} fibre analyser (Methods 13 and 12, respectively; Ankom Technology Corp., Macedon, NY, USA); the former was assayed with sodium sulphite and α-amylase, and both were expressed with residual ash. The content of ether extract in the diets was determined by the Ankom Filter Bag Technology (Method 2; Ankom Technology Corp.) and that of starch using a commercial kit (K-TSTA; Megazyme, Wicklow, Ireland). Fatty acid methyl esters (FAME) of lipid in feeds were prepared in a 1-step extraction-transesterification procedure (Shingfield et al., 2003), using *cis*-12 tridecenoate (Larodan Fine Chemicals AB, Malmö, Sweden) as an internal standard. Methyl esters were separated and quantified by gas chromatography (GC) using a temperature gradient program (Shingfield et al., 2003), and peaks were identified based on retention time comparisons with commercially available standard FAME mixtures (from Nu-Chek Prep., Elysian, MN, USA; and Sigma-Aldrich, Madrid, Spain).

Milk fat, protein, lactose and total solids contents were determined by infrared spectrophotometry (ISO 9622:1999). Lipid in 1 mL of milk was extracted and converted to FAME by base catalysed transesterification (Shingfield et al., 2003). Total FAME profile was determined by GC using the same temperature gradient program applied for the analysis of feeds, but isomers of 18:1 were further resolved in a separate analysis under isothermal conditions (Shingfield et al., 2003). Peaks were identified based on retention time comparisons with the same FAME mixtures used for the analysis of feeds, other commercially available standards (from Nu-Chek Prep.; Sigma-Aldrich; and Larodan Fine Chemicals AB), and comparison with reference samples for which the FA composition was determined based on GC analysis of FAME and GC-mass spectrometry analysis of corresponding 4,4-dimethyloxazoline derivatives (Bichi et al., 2013).

2.4. Calculations and statistical analysis

The mean milk fat melting point was estimated as the sum of the melting points of FA weighted by their respective molar proportions, as outlined in Toral et al. (2013).

All data were analysed by one-way analysis of variance using the MIXED procedure of the SAS software package (version 9.4, SAS Institute Inc., Cary, USA). The statistical model included the fixed effect of treatment (mean values over days 25 and 27 of experiment) and the initial record measured at the end of the adaptation period (mean values over days −3 and −1 for animal performance and day −1 for FA composition) as a covariate. Animals were nested within the treatment and used as the error term to contrast the effect of lipid supplementation. Significant differences were declared at $P<0.05$ and tendencies accepted if $P<0.10$. Means were separated through the “pdiff” option of the “lsmeans” statement of the MIXED procedure, and least squares means (adjusted for the covariance) are reported throughout.

3. Results

3.1. Diet composition and intake

The chemical composition of the experimental diets is reported in Table 1. By design, ingredients in the control diet were partially replaced by lipid supplements in FO and CLA treatments, increasing their ether extract content by approximately 89 and 48%, respectively.

Feed intake on the last week of the experiment averaged 3.11, 2.96 and 3.09 kg DM/day and ewe for the control, FO and CLA treatments, respectively. Supplementation with fish oil (Table 1) resulted in numerically higher daily intakes of 14:0, 16:0, *cis*-9 16:1, 18:0 and *cis*-9 and *cis*-11 18:1 than the control, and provided approx. 17 g/day of very long-chain n-3 FA (sum of 20:5n-3, 22:5n-3 and 22:6n-3). The rumen-protected CLA product included 16:0, 18:0, *cis*-9 18:1, and *cis*-9 *trans*-11 and *trans*-10 *cis*-12 18:2, supplying a daily dose of 40 mg *trans*-10 *cis*-12 18:2/kg BW.

3.2. Milk yield and composition

Feeding FO and CLA had no effect on individual milk yield (Table 2). However, compared with the control, both supplemented diets decreased ($P<0.05$; Figure 1) in a similar manner the contents of milk fat (–18%), protein (–13%) and lactose (–9%), and also tended to reduce milk fat production (–26%; $P=0.09$; Table 2).

No differences were detected between FO and CLA with regard to the contribution of major FA groups to MFD. Thus, on a molar basis, de novo-synthesized FA (<C16) accounted for 62% of the reduction in milk fat yield with both supplements, and C16 FA for another 27%. In contrast, variations in the yield of FA derived from plasma uptake (>C16), which averaged 10%, did not attain statistical significance ($P>0.10$; Figure 1).

3.3. Milk FA composition

As reported in Table 3, the CLA treatment had very limited influence on milk FA composition, other than increases ($P<0.001$) in *trans*-10 *cis*-12 18:2 and 18:0, reductions ($P<0.05$) in *cis*-9 10:1, *cis*-9 12:1 and 14:0 *iso*, and trends ($P<0.10$) towards greater 4:0 and lower 12:0 concentrations. In contrast, the FO diet significantly modified the proportion of most individual FA, particularly long-chain FA, with decreases in 18:0 and *cis*-FA (such as *cis*-9, *cis*-12 and *cis*-16 18:1 and *cis*-9 *cis*-12 18:2) and marked increases in 10-O-18:0, *trans*-FA (such as *trans*-11 and *trans*-12 18:1, *trans*-9 *cis*-12, *trans*-11 *cis*-15, *trans*-11 *trans*-15, *cis*-9 *trans*-11 and *trans*-9 *cis*-11 18:2), and 20- and 22-carbon unsaturated FA (such as 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3). Furthermore, 20:4n-3 and 22:2n-6 were only detected in the milk of ewes fed the marine lipid supplement. The content of *trans*-10 18:1 tended to increase in response to FO ($P=0.06$), but that of *trans*-10 *cis*-12 18:2 was not significantly different from the control ($P>0.10$). The effects of this marine oil on short- and medium-chain FA concentrations included increases in 6:0, 8:0 and *cis*-9 and *trans*-11 16:1 ($P<0.05$) as well as reductions in 12:0 and *cis*-9 12:1 ($P<0.10$; Table 3). In addition, the FO diet decreased the proportion of some milk odd- and branched-chain FA (namely, 14:0 *iso*, 15:0, 15:0 *anteiso*, 17:0, and 17:0 *anteiso*; $P<0.05$). Overall, these changes in milk FA profile due to FO (for additional information, please see Supplemental Table 1) decreased the estimated milk fat melting point compared with both the control and CLA treatments ($P<0.01$).

4. Discussion

Diet supplementation with rumen-protected CLA or fish oil decreased the content of milk fat to a similar extent (–18%). However, the observed reduction was lower than expected on the basis of previous reports in ewes fed similar doses of lipid-encapsulated CLA

(37-41 mg of *trans*-10 *cis*-12 18:2/kg BW; Lock et al., 2006; Sinclair et al., 2007) or a lower level of fish oil (1% diet DM; Toral et al., 2010a). This discrepancy might be linked to individual differences in responsiveness to MFD (Weimer et al., 2010).

Depending on the feeding system, reductions in energy requirements for milk fat synthesis due to MFD may allow for a repartitioning of nutrients towards increased milk yield and protein synthesis (Bauman et al., 2011). Nevertheless, consistent with our results, a lack of changes in milk yield is frequently observed during MFD (Sinclair et al., 2007; Toral et al., 2010b; Bichi et al., 2013). On the contrary, direct decreases in milk protein content (i.e., not linked to changes in milk yield) due to CLA supplementation are less common (Baumgard et al., 2000; Sinclair et al., 2007; Weerasinghe et al., 2012), which contrasts with the frequent reductions recorded in ewes on rations supplemented with fat sources (Mele et al., 2006; Toral et al., 2010a, 2010b). Although the effect of lipid supplements on mammary protein metabolism has been related to nutritional and endocrine factors, including decreased amino acid availability and insulin resistance (DePeters and Cant, 1992; Mackle et al., 2000), the available data are too limited to be conclusive and further research into this subject is still needed. Similarly, consistent with studies in dairy cows and ewes (Bell and Kennelly, 2006; Sinclair et al., 2007), CLA supplementation decreased milk lactose concentration, the reasons for this response being still uncertain.

The syndrome of MFD has been consistently associated with alterations in microbial lipid metabolism in the rumen that favour the formation of specific BH intermediates with potential antilipogenic effects (Shingfield and Griinari, 2007). Thus, although theories relying on other bases (such as the glucogenic-insulin theory or those related to an insufficient supply of precursors for mammary milk fat synthesis) have been proposed, they have been subsequently found inadequate (Bauman and Griinari, 2001). The changes in milk FA composition observed in this study would allow to infer that FO affected the lipid metabolism

in the rumen and induced MFD without promoting an increment in *trans*-10 *cis*-12 18:2, in agreement with earlier reports in ewes and cows (Shingfield et al., 2003; Toral et al., 2010a; Kairenius et al., 2015). Conversely, FO supplementation enhanced milk concentrations of other FA that have also been tentatively related to MFD, namely, *trans*-10 18:1 and *trans*-9 *cis*-11 18:2 (Shingfield and Griinari, 2007), but previous studies in ewes fed oil-supplemented diets were not able to associate MFD with similar or even higher percentages of these FA in milk (Gómez-Cortés et al., 2008; Toral et al., 2010a).

Therefore, it appears reasonable to suspect that other less well-known BH intermediates may be involved in the low-fat milk syndrome (Shingfield and Griinari, 2007). Although the negative effect of FO on *trans*-18:1 hydrogenation and the shift towards the formation of *trans*-10 18:1 at the expense of *trans*-11 18:1 have received a great deal of attention, additional BH steps and pathways could also be altered by marine lipids and promote the formation of other candidate antilipogenic FA. In this respect, Alves and Bessa (2014) reported large increases in the ruminal concentration of *trans*-10 *cis*-15 18:2 (a candidate milk fat inhibitor) during the "*trans*-10 shift", which would suggest alterations in the early stages of 18:3n-3 BH. Since, according to Kairenus et al. (2015), *trans*-10 *cis*-15 18:2 would coelute with *trans*-11 *cis*-15 18:2 under our chromatographic conditions, it is speculated that marked increases in the latter in response to FO supplementation would have most likely been accompanied by increments in *trans*-10 *cis*-15 18:2. Regardless, further research is necessary to prove the antilipogenic action of this isomer, either with its abomasal or intravenous administration or through in vitro cultures of mammary epithelial cells.

Studies in dairy cows have also shown that fish oil may decrease milk fat concentration when infused post-ruminally (Loor et al., 2005; Dallaire et al., 2014), suggesting a putative antilipogenic effect of some constituent of this lipid supplement. In this respect, 20:5n-3 was shown to downregulate the expression of mammary lipogenic genes in vitro (Kadegowda et

al., 2009) and *cis*-9 16:1 can negatively affect adipogenesis (Burns et al., 2012; Duckett et al., 2014). However, even though the actual role of both FA in MFD merits further investigation, the large difference in the reduction of milk fat concentration caused by fish oil infusion either ruminally or post-ruminally (Loor et al., 2005) provides evidence that its constituents would not be major responsible for the low-fat milk syndrome. This observation would point again to the involvement of microbial BH intermediates (which may derive not only from the fish oil FA metabolism but also from that of other diet ingredients).

Thus, it is also worth noting the increase in milk 10-oxo-18:0, which results from the sequential hydration and oxidation of unsaturated FA in the rumen (Jenkins et al., 2006). Although the content of keto-FA is rarely reported in milk, these components might have bioactive effects, including a potential action on mammary lipogenesis related to the oxo group located on carbon 10 (Kairenius et al., 2015). In addition, increases with FO, which agree with observations in sheep and cows (Bichi et al., 2013; Kairenius et al., 2015), would indicate alternative pathways to BH that may point to more global changes in the microbial lipid metabolism in the rumen, thereby providing further explanations of MFD (Shingfield and Griinari, 2007). Variations in milk concentrations of odd- and branched-chain FA in response to FO would also suggest alterations in the rumen bacterial community (Fievez et al., 2012), probably due to the toxic effects of unsaturated FA on microbiota (Maia et al., 2007; Castro-Carrera et al., 2014), which might have an impact on the composition of FA available for mammary uptake and be at the core of the associated MFD.

In line with this, marine lipids are known to induce a shift in milk from *cis*-9 18:1 to *trans* 18:1, which might increase the mean melting point of the fat and exceed the upper limit for the maintenance of milk fat fluidity at body temperature. This fact has been proposed to result in MFD (Shingfield and Griinari, 2007), even though the actual relevance of this extension of the BH theory has not yet been well established. In fact, the decrease in the

estimated milk fat melting point in the FO treatment, together with the lack of variation in this value in some previous studies (Toral et al., 2010b, 2013; Kairenius et al., 2015), would support that the mammary gland is capable of adapting to substantial changes in the pool of FA available for milk fat synthesis, without apparent increases in the mean melting point that could impair the rate of milk lipid secretion. However, these observations do not exclude the possibility of an accumulation of milk FA with high melting points in mammary epithelial cells, given that samples for lipid analysis are only collected from milk that has been successfully secreted (Gama et al., 2008). Further work is then necessary to test the actual role of milk fat fluidity regulation in marine lipid-induced MFD.

Comparison of the changes in the molar production of milk FA showed that de novo FA synthesis was affected similarly in FO and CLA treatments and more strongly than FA uptake, which implies that both types of MFD might share common mechanisms. This finding is consistent with results based on indirect comparison between *trans*-10 *cis*-12 18:2- (Lock et al., 2006; Sinclair et al., 2007) and marine lipid- (Toral et al., 2010a, 2010b; Bichi et al., 2013) induced MFD in dairy ewes, although it must be mentioned that most observations about the latter derive from studies in which the dietary supplements included not only marine but also plant lipids. On the other hand, contrasting results have been obtained in dairy cows (Shingfield et al., 2003; Rego et al., 2005; Pirondini et al., 2015), with some reports showing that MFD caused by marine lipid was characterized by a greater reduction in the molar yield of milk long-chain FA than in that of short- and medium-chain FA.

5. Conclusions

The direct comparison of milk FA profiles in ewes supplemented with either FO or rumen-protected CLA support that marine lipid-induced MFD is not mediated by the effects of *trans*-10 *cis*-12 18:2. However, changes in the molar yield of milk FA deriving from de

novo synthesis or uptake from plasma suggest that both types of MFD could share common mechanisms. The results point to the involvement of less well-known potentially antilipogenic metabolites (such as intermediates of 18:3n-3 BH or ruminal hydration and oxidation) in the low-milk fat syndrome in ewes fed FO and seem to downplay the relevance of changes in the milk fat melting point as a major mechanism responsible for this.

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FIGURE CAPTION

Figure 1

Milk fat content and fatty acid (FA) yield in ewes fed a diet without supplementation (control; □) or supplemented with 2% DM of fish oil (FO; ▫) or 1.1% DM of a product rich in *trans*-10 *cis*-12 18:2 (CLA; ■). Vertical bars represent the standard error of the difference for treatment effects.

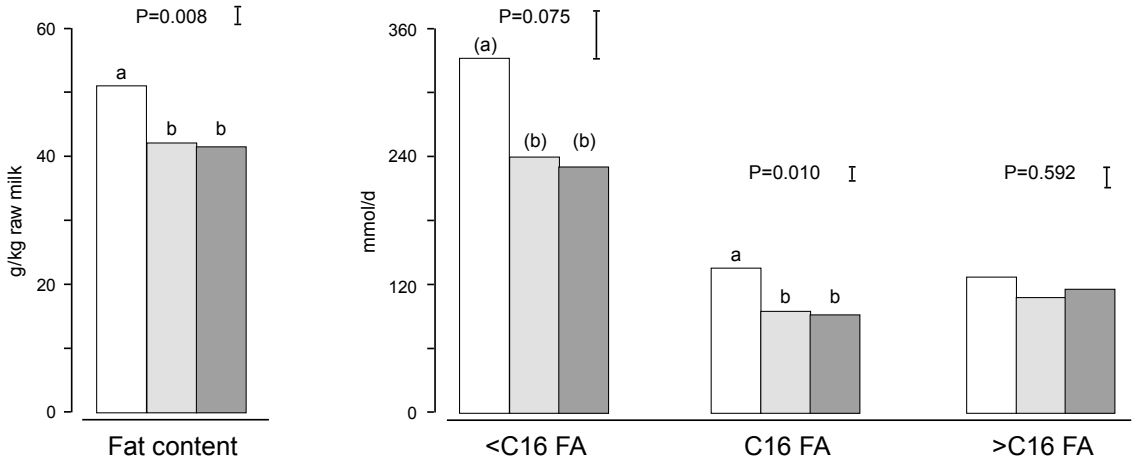

Table 1

Formulation and chemical composition of the experimental diets and daily fatty acid intake in ewes fed the total mixed ration without supplementation (control) or supplemented with 2% DM of fish oil (FO) or 1.1% DM of a product rich in *trans*-10 *cis*-12 18:2 (CLA).

	Treatment		
	Control[A]	FO	CLA
Ingredients, g/kg of fresh matter			
Dehydrated alfalfa hay	400	393	396
Whole corn grain	180	177	178
Whole barley grain	130	128	129
Soybean meal solvent 440 g CP/kg	150	147	149
Sugar beet pulp, pellets	70	69	69
Molasses, liquid	50	49	50
Fish oil[B]	0	18	0
Rumen-protected CLA product[C]	0	0	10
Mineral supplement[D]	18	18	18
Vitamin supplement[E]	2	2	2
Chemical composition, g/kg DM			
Organic matter	914	902	904
Crude protein	186	186	185
Neutral detergent fibre	231	238	245
Acid detergent fibre	147	147	150
Starch	222	219	225
Ether extract	22.7	42.8	33.7
Fatty acid intake, g/d			
14:0	0.761	2.682	0.756
16:0	13.4	24.1	15.9
cis-9 16:1	0.198	2.68	0.197
18:0	2.92	6.14	16.7
cis-9 18:1	9.57	18.7	13.8
cis-11 18:1	0.635	2.44	0.630
cis-9 *trans*-11 18:2	0	0	2.86
trans-10 *cis*-12 18:2	0	0	2.94
18:2n-6	27.2	27.1	27.2
18:3n-3	6.69	6.85	6.65
20:5n-3	0	3.55	0
22:5n-3	0	0.891	0
22:6n-3	0	12.7	0

[A] Contained (g/kg total FA) 14:0 (11.6), 16:0 (204), 18:0 (44.7), *cis*-9 18:1 (146), *cis*-11 18:1 (9.70), 18:2n-6 (417) and 18:3n-3 (102).

[B] Semirefined tuna and sardine oil (Afampes 121 DHA; Afamsa, Mos, Spain); contained (g/kg total FA) 14:0 (33.1), 16:0 (194), *cis*-9 16:1 (42.0), 17:0 (8.93), 18:0 (56.8), *cis*-9 18:1

(163), *cis*-11 18:1 (31.1), 18:2n-6 (21.2), 18:3n-3 (8.35), *cis*-11 20:1 (16.4), 20:5n-3 (60.0), 22:5n-3 (15.1) and 22:6n-3 (214).

[C] Lutrell Pure (BASF, Ludwigshafen, Germany); contained (g/kg total FA) 16:0 (97.5), 18:0 (501), *cis*-9 18:1 (155), *cis*-9 *trans*-11 18:2 (104) and *trans*-10 *cis*-12 18:2 (107) and 807 g total FA/kg DM.

[D] Declared as containing (g/kg): $CaCO_3$ (556), Ca_2HPO_4 (222) and NaCl (222).

[E] *VITAFAC Ovino 0.2% AC* (DSM Nutritional Products S.A., Madrid, Spain). Declared as containing: vitamin A (4,000,000 IU/kg), vitamin D3 (1,000,000 IU/kg), vitamin E (5 g/kg), iron (17.5 g/kg), manganese (20 g/kg), cobalt (50 mg/kg), iodine (250 mg/kg), zinc (15 g/kg), selenium (100 mg/kg), sepiolite (100 g/kg), calcium (26.2 g/kg) and magnesium (6.15 g/kg).

Table 2

Milk yield and composition in ewes fed the total mixed ration without supplementation (control) or supplemented with 2% DM of fish oil (FO) or 1.1% DM of a product rich in *trans*-10 *cis*-12 18:2 (CLA).

	Treatment				
	Control	FO	CLA	SED[A]	P[B]
Yield, g/d					
Milk	2,724	2,434	2,535	322	0.659
Fat	139	103	104	16.4	0.090
Protein	149	113	114	17.2	0.094
Lactose	136	118	115	17.2	0.447
Total solids	447	350	355	51.5	0.152
Composition, g/kg					
Fat	51.0[a]	42.2[b]	41.6[b]	2.48	0.008
Protein	53.4[a]	47.5[b]	44.9[b]	1.99	0.003
Lactose	50.0[a]	47.9[ab]	45.5[b]	1.26	0.019
Total solids	163[a]	145[b]	140[b]	4.37	0.001

[a-b] Within a row, different superscripts indicate significant differences ($P<0.05$).

[A] SED = standard error of the difference for treatment effects.

[B] Probability of significant effects due to experimental treatment.

Table 3

Milk fatty acid (FA) composition in ewes fed the total mixed ration without supplementation (control) or supplemented with 2% DM of fish oil (FO) or 1.1% DM of a product rich in *trans*-10 *cis*-12 18:2 (CLA) (effects on additional FA are reported in Supplemental Table S1).

	Treatment				
FA, g/100 g total FA	Control	FO	CLA	SED[A]	P[B]
Saturated FA					
4:0	2.68	2.74	3.17	0.206	0.074
6:0	2.62[b]	3.07[a]	2.62[b]	0.143	0.026
8:0	2.88[b]	3.64[a]	2.84[b]	0.248	0.033
10:0	11.5	11.7	10.0	0.881	0.200
12:0	7.57	6.83	6.41	0.516	0.087
14:0	13.2	12.2	13.1	0.597	0.260
14:0 *iso*	0.120[a]	0.059[c]	0.095[b]	0.0074	<0.001
15:0	1.11[a]	0.824[b]	1.02[ab]	0.0883	0.031
15:0 *iso*	0.154	0.130	0.188	0.0241	0.112
15:0 *anteiso*	0.462[a]	0.304[b]	0.425[a]	0.0316	0.004
16:0	25.3	24.6	23.2	1.21	0.290
17:0	0.575[a]	0.452[b]	0.594[a]	0.0355	0.010
17:0 *iso*	0.501	0.621	0.558	0.0494	0.108
17:0 *anteiso*	0.486[a]	0.367[b]	0.491[a]	0.0401	0.018
18:0	6.51[b]	1.14[c]	8.56[a]	0.423	<0.001
10-oxo-18:0	0.001[b]	0.461[a]	0.011[b]	0.0807	<0.001
Monounsaturated FA					
cis-9 10:1	0.280[a]	0.280[a]	0.181[b]	0.0165	<0.001
cis-9 12:1	0.133[a]	0.094[b]	0.076[b]	0.0099	0.001
cis-9 14:1	0.204	0.165	0.183	0.0230	0.286
cis-9 16:1	0.744[b]	1.13[a]	0.698[b]	0.0735	0.001
trans-9 16:1	0.091[b]	0.456[a]	0.130[b]	0.0385	<0.001
cis-9 18:1[C]	10.1[a]	6.63[b]	10.7[a]	0.660	<0.001
cis-11 18:1	0.849	1.17	0.956	0.158	0.190
cis-12 18:1	0.342[a]	0.141[b]	0.355[a]	0.0435	0.003
cis-13 18:1	0.093	0.130	0.088	0.0231	0.179
cis-16 18:1	0.052[a]	0.022[b]	0.065[a]	0.0069	0.001
trans-9 18:1	0.270	0.329	0.272	0.0534	0.469
trans-10 18:1	0.377	1.41	0.795	0.374	0.060
trans-11 18:1	1.20[b]	4.32[a]	1.71[b]	1.02	0.002
trans-12 18:1	0.365[b]	0.868[a]	0.384[b]	0.0607	<0.001
trans-15 18:1[D]	0.713[a]	0.514[b]	0.768[a]	0.0507	0.004
Σ 20:1 + 22:1	0.139[b]	0.671[a]	0.119[b]	0.0500	0.002
Polyunsaturated FA					
cis-9 *cis*-12 18:2	2.46[a]	1.74[b]	2.53[a]	0.242	0.029
trans-9 *cis*-12 18:2	0.023[b]	0.102[a]	0.034[b]	0.0207	0.014
trans-11 *cis*-15 18:2	0.053[b]	0.458[a]	0.092[b]	0.0465	<0.001
trans-11 *trans*-15 18:2	0.010[b]	0.049[a]	0.021[b]	0.0080	0.005
cis-9 *trans*-11 18:2[E]	0.497[b]	2.44[a]	0.718[b]	0.206	<0.001

trans-9 *cis*-11 18:2	0.024[b]	0.129[a]	0.037[b]	0.0144	<0.001
trans-10 *cis*-12 18:2	0.014[b]	0.032[b]	0.090[a]	0.0113	<0.001
Σ other *trans,trans* conjugated 18:2[F]	0.062	0.074	0.080	0.0123	0.346
cis-9 *cis*-12 *cis*-15 18:3	0.494	0.437	0.528	0.0601	0.357
cis-11 *cis*-14 *cis*-17 20:3	0.005[b]	0.044[a]	0.003[b]	0.0149	0.048
cis-8 *cis*-11 *cis*-14 *cis*-17 20:4	nd[G]	0.019	nd	-	-
cis-5 *cis*-8 *cis*-11 *cis*-14 *cis*-17 20:5	0.038[b]	0.490[a]	0.065[b]	0.0516	<0.001
cis-13 *cis*-16 22:2	nd	0.034	nd	-	-
cis-7 *cis*-10 *cis*-13 *cis*-16 *cis*-19 22:5	0.067[b]	0.559[a]	0.089[b]	0.0274	<0.001
cis-4 *cis*-7 *cis*-10 *cis*-13 *cis*-16 *cis*-19 22:6	0.035[b]	1.34[a]	0.032[b]	0.0929	<0.001
Estimated milk fat melting point (ºC)	36.9[a]	33.8[b]	36.8[a]	0.668	0.001

[a-c] Within a row, different superscripts indicate significant differences (P<0.05).

[A] SED = standard error of the difference for treatment effects.

[B] Probability of significant effects due to experimental treatment.

[C] Coelutes with *trans*-13+14 18:1.

[D] Contains *cis*-10 18:1 as minor component.

[E] Contains *trans*-8 *cis*-10 and *trans*-7 *cis*-9 18:2 as minor components.

[F] Sum of *trans*-8 *trans*-10, *trans*-9 *trans*-11, *trans*-10 *trans*-12 and *trans*-11 *trans*-13 18:2.

[G] Not detected.

Supplemental Table 1

Other fatty acids (FA) in milk from ewes fed the total mixed ration without supplementation (control) or supplemented with 2% DM of fish oil (FO) or 1.1% DM of a product rich in *trans*-10 *cis*-12 18:2 (CLA).

	Treatment				
FA, g/100 g total FA	Control	FO	CLA	SED[A]	P[B]
Saturated FA					
5:0	0.023	0.019	0.022	0.0035	0.544
7:0	0.047	0.040	0.038	0.0061	0.301
9:0	0.099	0.078	0.076	0.0113	0.112
11:0	0.180	0.116	0.132	0.2614	0.088
13:0	0.164	0.093	0.127	0.0246	0.064
13:0 *iso*	0.017	0.020	0.016	0.0029	0.392
13:0 *anteiso*	0.013	0.011	0.008	0.0025	0.144
16:0 *iso*	0.251	0.191	0.208	0.0372	0.307
4,8,12-trimethyl-13:0	0.111	0.129	0.108	0.0137	0.290
18:0 *iso*	0.061	0.055	0.053	0.0068	0.507
20:0	0.227[a]	0.138[b]	0.247[a]	0.0321	0.024
21:0	0.055	0.056	0.055	0.0068	0.975
22:0	0.072	0.086	0.080	0.0106	0.423
23:0	0.049	0.115	0.043	0.0280	0.065
24:0	0.026[b]	0.047[a]	0.026[b]	0.0070	0.020
Monounsaturated FA					
trans-9 12:1	0.058[a]	0.043[b]	0.039[b]	0.0057	0.017
cis-7 14:1	0.029	0.021	0.029	0.0069	0.466
cis-12 14:1	0.124[a]	0.089[b]	0.074[b]	0.0122	0.008
trans-5+6 14:1	0.031	0.034	0.036	0.0068	0.734
trans-9 14:1	0.012	0.008	0.011	0.0027	0.306
trans-5+6+7 15:1	0.121	0.159	0.132	0.212	0.250
cis-11 16:1	0.038[b]	0.063[a]	0.044[b]	0.0053	0.002
cis-14 16:1[C]	0.215[a]	0.172[ab]	0.141[b]	0.0215	0.022
cis-9 17:1	0.225	0.215	0.197	0.0216	0.416
trans-4 18:1	0.021	0.020	0.026	0.0037	0.231
trans-5 18:1	0.007[b]	0.017[a]	0.013[a]	0.0024	0.012
trans-6+7+8 18:1	0.184	0.173	0.159	0.0572	0.865
trans-16 + *cis*-14 18:1	0.464	0.318	0.447	0.0727	0.169
cis-9 20:1	0.048	0.087	0.042	0.0247	0.216
cis-11 20:1	0.037[b]	0.349[a]	0.056[b]	0.0340	<0.001
cis-13 22:1	0.016[b]	0.091[a]	0.007[b]	0.0157	0.002
trans-13 22:1	0.020	0.049	0.015	0.0018	0.184
cis-15 24:1	0.005[b]	0.023[a]	0.005[b]	0.0051	0.018
Polyunsaturated FA					
cis-9 *trans*-12 18:2	0.059	0.087	0.057	0.0168	0.226
cis-9 *trans*-13 18:2	0.177	0.195	0.169	0.0331	0.724
cis-9 *trans*-14 18:2	0.071[a]	0.031[b]	0.066[a]	0.0076	0.003
trans-9 *trans*-12 18:2	0.064	0.099	0.086	0.0192	0.206

Δ10,14 18:2	0.081	0.118	0.105	0.0147	0.095
cis-11 *cis*-14 20:2	0.028[b]	0.098[a]	0.025[b]	0.0112	<0.001
cis-8 *cis*-11 *cis*-14 20:3	0.023[b]	0.063[a]	0.023[b]	0.0060	<0.001
cis-7 *cis*-10 *cis*-13 *cis*-16 22:4	0.153[b]	0.369[a]	0.167[b]	0.0370	0.001
cis-8 *cis*-11 *cis*-14 20:3	0.015[b]	0.088[a]	0.015[b]	0.0056	<0.001
cis-5 *cis*-8 *cis*-11 *cis*-14 20:4	0.077[b]	0.195[a]	0.045[b]	0.0235	<0.001

[a-b] Within a row, different superscripts indicate significant differences (P<0.05).

[A] SED = standard error of the difference for treatment effects.

[B] Probability of significant effects due to experimental treatment.

[C] Coelutes with 3,7,11,15-tetramethyl-16:0.

HIGHLIGHTS:

1. Milk fat depression (MFD) induced by fish oil or *t*10*c*12-18:2 was compared in ewes

2. Fish oil-induced MFD was not mediated by the antilipogenic effect of *t*10*c*12-18:2

3. Less well-known antilipogenic fatty acids may be involved in fish oil-induced MFD in ewes

4. Molar yield data suggest that both types of MFD might share common mechanisms

5. Results seem to downplay the relevance of changes in milk fat melting point in MFD