Dietary sunflower oil modulates milk fatty acid composition without major changes in adipose and mammary tissue fatty acid profile and related gene mRNA abundance in sheep

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Short title: Tissue lipid metabolism in sheep fed sunflower oil

Abstract

There are very few studies in ruminants characterizing mammary and adipose tissue (AT) expression of genes and gene networks for diets causing variations in milk fatty acid (FA) composition without altering milk fat secretion, and even less complementing this information with data on tissue FA profiles. This work was conducted in sheep to investigate the response of the mammary gland and the subcutaneous and perirenal AT, in terms of FA profile and mRNA abundance of genes involved in lipid metabolism, to a diet known to modify milk FA composition.

Ten lactating Assaf ewes were randomly assigned to 2 treatments consisting of a total mixed ration based on alfalfa hay and a concentrate (60:40) supplemented with 0 (control diet) or 25 (SO diet) g of sunflower oil/kg of diet DM for 7 weeks. Milk composition, including FA profile, was analysed after 48 days on treatments. On day 49, the animals were euthanized and tissue samples were collected to analyse FA and mRNA abundance of 16 candidate genes. Feeding SO did not affect animal performance but modified milk FA composition. Major changes included decreases in the concentration of FA derived from *de novo* synthesis (e.g., 12:0, 14:0 and 16:0) and increases in that of long chain FA (e.g., 18:0, c9-18:1, *trans*-18:1 isomers and c9,t11-CLA) but they were not accompanied by significant variations in the mRNA abundance of studied lipogenic genes (i.e., *ACACA*, *FASN*, *LPL*, *CD36*, *FABP3*, *SCD1* and *SCD5*) and transcription factors (*SREBF1* and *PPARG*), or in the constituent FA of mammary tissue. Regarding the FA composition of AT, the little influence of SO did not appear to be linked to changes in gene mRNA abundance (decreases of *GPAM* and *SREBF1* in both tissues, and of *PPARG* in the subcutaneous depot). Similarly, the great variation between AT (higher contents of saturated FA and *trans*-18:1 isomers in the perirenal, and of *cis*-18:1, c9,t11-CLA and n-3 PUFA in the subcutaneous AT) could not be related to differences in gene mRNA abundance due to tissue site (higher *LPL* and *CD36*, and lower *SREBF1* in perirenal than in subcutaneous AT). Overall, these results suggest a marginal contribution of gene expression to the nutritional regulation of lipid metabolism in these tissues, at least with the examined diets and after 7 weeks on treatments. It cannot be ruled out, however, that the response to SO is mediated by other genes or post-transcriptional mechanisms.

Keywords: fatty acid composition, lactating ewe, lipogenic gene expression, plant oil, tissue

Implications

A better knowledge of the molecular mechanisms underlying mammary lipogenesis may help us to understand, and eventually modulate, the effect of nutrition on milk fat production and quality. This work was conducted in dairy sheep to investigate if responses in milk fatty acid composition to dietary linoleic-rich oils could be related to tissue mRNA abundance of genes involved in lipid metabolism. The study was carried out with ewes fed a diet supplemented with sunflower oil for 7 weeks, and shows that effects on milk fatty acid profile were not accompanied by major changes in either fatty acid composition or mRNA abundance of lipogenic genes in mammary or adipose tissue.

Introduction

The addition of a moderate amount of sunflower oil (**SO**) to a forage-rich diet has proved in dairy ewes to modulate milk fat composition without negatively affecting its production (Toral *et al.*, 2010; Gómez-Cortés *et al.*, 2011). However, mechanisms explaining changes in milk fatty acids (**FA**), including mediation via coordinated down- and up-regulation of genes involved in lipid metabolism, are still poorly understood. In addition, there are very few studies characterizing mammary expression of genes and gene networks for diets causing variations in milk FA composition without altering milk fat secretion (Shingfield *et al*., 2010). The available information is almost limited to cows suffering milk fat depression (**MFD**; Piperova *et al.*, 2000; Peterson *et al.*, 2003) or to goats (Bernard *et al.*, 2005a, 2005b and

2009a), a species in which this syndrome is not often observed, but little work has been published yet on ewes (Dervishi *et al*., 2012; Bichi *et al*., 2013).

Whereas the physiological adaptations of the mammary gland or adipose tissues (**AT**) occurring during lactation have been well described in ruminants (Vernon, 1980; Chilliard, 1999; Bauman, 2000), the possible contribution of AT to the regulation of mammary lipogenesis still needs to be documented (Shingfield *et al*., 2010). Studies on diet-induced MFD in cows report an increase in the mRNA abundance of lipogenic genes in AT related to the preferential partitioning of nutrients towards non-mammary tissues (Harvatine *et al.*, 2009; Thering *et al.*, 2009; Schmitt *et al.*, 2011). On the contrary, lipid supplementation inducing no changes or increases in milk fat yield in goats is not accompanied by alterations of the mRNA abundance or activity of lipogenic enzymes in AT (Bernard *et al.*, 2005a and 2009a). Although it is complicated to discern whether these differences are linked to the diet, its interaction with species, or other factors, there is no information at all in sheep.

Furthermore, most works relied on the study of subcutaneous AT (Harvatine *et al.*, 2009; Thering *et al*., 2009; Bichi *et al*., 2013), despite differences among AT in FA composition, lipogenic gene expression or enzyme activity have been described (Chilliard *et al*., 1981; Bas *et al*., 1987; Barber *et al*., 2000). Data on the FA profile of AT could complement results on gene mRNA abundance to improve our understanding of lipid metabolism, but simultaneous measurements are seldom reported (Toral *et al.*, 2013). Similarly, most studies last for up to only 4 weeks (Piperova *et al*., 2000; Bernard *et al*., 2009b; Jacobs *et al*., 2011) and mechanisms involved in a longer term response are not considered. On the other hand, according to previous results in dairy ewes (e.g., Toral *et al*., 2010; Gómez-Cortés *et al.*, 2011), the longer the time on lipid-supplemented diets, the greater the differences in milk fat

composition. Therefore, it may be expected that changes in the mRNA abundance of genes involved in lipid metabolism would also be more easily observed after relatively long periods on lipid-treatments.

Thus, an assay with lactating ewes receiving SO for 7 weeks was conducted on the hypothesis that SO-induced changes in milk FA profile would be mediated by variations in mammary lipogenic gene expression, this response being also putatively related to changes in AT.

Materials and methods

Animals, experimental diets and management

Ten multiparous Assaf ewes (84.1 kg BW, s.d. 8.35; 75 days in milk, s.d. 3.7) were divided into two groups (n=5), balanced for milk yield, BW, days in milk, and number of lactation, and assigned to one of two experimental treatments (diets): control and supplemented with SO. Diets, prepared weekly, consisted of a total mixed ration (**TMR**) based on dehydrated alfalfa hay (particle size>4 cm) and a concentrate (60:40) supplemented with 0 (control diet) or 25 (SO diet) g of SO/kg of diet dry matter (**DM**). The ingredients and chemical composition of the experimental diets, which included molasses to reduce selection of dietary components, are given in Table 1. Clean water was always available and fresh diets were offered daily *ad libitum* at 0900 and 1900 h.

Ewes were milked twice daily at approximately 0830 and 1830 h in a 1x10 stall-milking parlour (DeLaval, Madrid, Spain). The experiment lasted for 7 weeks and was carried out in accordance with the Spanish Royal Decree 1201/2005 for the protection of animals used for experimental purposes.

Measurements and sampling procedures

Representative samples of offered and refused diets were collected weekly, stored at −20ºC and then freeze-dried. Diet samples were analyzed for DM, ash, CP, NDF, ADF and ether extract as outlined in Toral *et al*. (2010).

On day 48 of the experiment, individual milk yield was recorded both at morning and evening milkings, and milk samples were collected from each animal and stored at 4ºC with a preservative (bronopol) until analyzed for fat, CP, lactose and total solids by infrared spectrophotometry as described in Toral *et al*. (2010). Milk fat composition was determined in unpreserved samples stored at −30ºC.

At the end of the experiment (day 49), the ewes were slaughtered humanely by an intravenous injection of a euthanasia drug (T-61, Intervet, Salamanca, Spain; 0.1 mL/kg BW) and samples of the mammary secretory tissue, and subcutaneous (abdomen) and perirenal AT were collected under sterile conditions, immediately frozen in liquid N_2 and stored at –80ºC until lipid and RNA extraction.

Lipid analysis

Fatty acid methyl esters (FAME) of lipid in TMR and milk were prepared in a one-step extraction-transesterification procedure, as outlined previously by Shingfield *et al.* (2003). Total lipids in 500 mg of mammary secretory tissue and in 30 mg of subcutaneous and perirenal AT were extracted (Folch *et al.*, 1957), and converted to FAME using a base-acid catalyzed transesterification procedure (Glass, 1971). Methyl esters were separated and quantified by gas chromatography using a temperature gradient programme, and 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 authentic standards (from Nu-Chek Prep., Elysian, MN, USA; Sigma–Aldrich, Madrid, Spain; and Larodan Fine Chemicals AB, Malmö, Sweden), and milk samples for which the FA composition

was determined based on gas chromatography analysis of FAME and GC-MS analysis of corresponding 4,4-dimethyloxazoline derivatives (Shingfield *et al.*, 2006).

RNA isolation and quantitative PCR

Total RNA from each tissue was isolated and purified further as described in Bernard *et al.* (2005b). Concentration and purity of RNA was determined by spectrophotometry using a Nano-Drop ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA). The RNA integrity was evaluated using the Agilent Bioanalyzer system (Agilent Technologies, Santa Clara, CA, USA), the RIN value for all the samples being 7.2 ± 0.14. Reverse transcription qPCR was carried out (Bernard *et al.*, 2005b) using specific primers and probes (Supplementary Table S1) for the following genes: acetyl-CoA carboxylase alpha (*ACACA*) and fatty acid synthase (*FASN*), involved in *de novo* FA synthesis; lipoprotein lipase (*LPL*), thrombospondin receptor or fatty acid translocase (*CD36*) and fatty acid-binding proteins 3 (*FABP3*) and 4 (*FABP4)*, involved in the uptake and intracellular transport of FA; stearoyl-CoA desaturase (*SCD1* and *SCD5*), involved in Δ9-desaturation of FA; glycerol-3-phosphate acyltransferase (*GPAM*), involved in the esterification of FA to glycerol; carnitine palmitoyl transferase 2 (*CPT2*), involved in the mitochondrial oxidation of FA; fatty acid elongases 5 and 6 (*ELOVL5* and *ELOVL6*), involved in the elongation to very long-chain and long-chain FA respectively; milk fat globule-EGF factor 8 (*MFGE8*) and xanthine dehydrogenase (*XDH*), encoding two of the major proteins of the milk fat globule membrane; and the transcription factors: sterol regulatory element binding transcription factor 1 (*SREBF1*) and peroxisome proliferator-activated receptor gamma (*PPARG*). To account for variations in RNA integrity, RNA quantification and cDNA synthesis, mRNA abundance was normalized using the geometric mean of three housekeeping genes: peptidylprolyl isomerase A

(*PPIA*), ubiquitiously-expressed transcript (*UXT*) and eukaryotic translation initiation factor 3 subunit K (*EIF3K*), which were identified as suitable internal controls among several tested (Bonnet *et al.*, 2013). Abundance of gene transcripts was expressed as the log value of mRNA copy number relative to the geometric mean of the three housekeeping genes.

Statistical analysis

All statistical analyses were performed using the MIXED procedure of the SAS software package (version 9.3, SAS Inst. Inc., Cary, NC, USA). Data of diet composition, animal performance, milk and mammary FA profiles, as well as mammary mRNA abundances of candidate genes were analysed by a one-way ANOVA with a statistical model that included the fixed effect of the diet. Two-way ANOVA was applied to data of FA composition and gene mRNA abundance of AT, the statistical model including the fixed effects of the diet, the adipose site and their interaction. In both cases, animals were nested within the treatment and used as the error term to contrast the effect of SO supplementation. Differences between means were evaluated using the 'pdiff option' of the 'LS means' statement of the MIXED procedure. They were declared significant at $P<0.05$ and considered as tendencies toward significance at $P<0.10$. Least square means are reported throughout.

Results

Animal performance and milk FA composition and secretion

The inclusion of SO did not affect any of the animal performance parameters studied (i.e. DM intake, calculated energy balance, milk yield and milk fat, protein, lactose and total solid composition and yields; Table 2; $P>0.10$).

In contrast, as shown in Table 3 and Supplementary Table S2, milk FA profile

changed noticeably. The dietary addition of SO decreased milk concentration of <16 and 16-carbon FA and increased that of >16-carbon FA (P<0.01). The proportion of 18:0, some 18:1 intermediates, such as c9-18:1, t11-18:1 and t10-18:1, and some conjugated linoleic acid (**CLA**) isomers (e.g., c9,t11-CLA) was augmented in response to dietary SO (P<0.05). Lipid supplementation also caused significant variations in other milk FA, such as decreases in 12:0, 14:0, 16:0, c9-17:1, t11,c15-18:2 and 18:3n-3, and increases in most minor 18:2 isomers.

The effect of dietary treatment on milk FA secretion is presented in Table 4. The inclusion of SO significantly augmented the yield of >16-carbon FA, while no effect was detected on 16-carbon, and <16-carbon FA tended to be reduced (P<0.10). Reflecting the effects reported for milk FA concentrations, the secretion of 18:0, some 18:1 isomers, and total CLA (particularly c9,t11-CLA) was higher with the SO diet. On the other hand, SO supplementation did not affect saturates, odd- and branched-chain FA (**OBCFA**) and PUFA n-6 secretion (P>0.10).

Mammary and adipose tissue FA composition

Most of the diet-induced differences observed in milk FA composition were not detected in the mammary secretory tissue (Table 3 and Supplementary Table S3), where changes due to SO were limited to a tendency (P<0.10) to a reduction of some OBCFA (17:0, anteiso-17:0 and iso-18:0) and a rise in some *trans* isomers (t5- to t9-18:1 and t9,c12-18:2). Major differences in the FA composition of AT were related to the effect of tissue site rather than to dietary treatment (Table 5 and Supplementary Table S4). The subcutaneous AT was richer in OBCFA, unsaturated FA, especially Δ9-desaturase products, other *cis*-18:1 and CLA isomers, and 20- and 22-carbon PUFA n-3 and n-6 (P<0.05), while the perirenal AT showed a higher content of 18:0 and *trans*-18:1 (P<0.01). Some of the few differences in FA profile due to dietary

treatments (P<0.05) were in line with those mentioned for the mammary tissue (e.g., the decrease in 17:0 and 18:3n-3). Significant interactions between the effects of tissue site and diet were only observed for minor FA (namely t9,c12,c15-18:3 and iso-16:0; Table 5).

mRNA abundances of candidate genes in mammary and adipose tissues

The mRNA abundance of some candidate genes (Tables 6, 7 and S5) was tissue-specific: *MFEG8*, *XDH* and *FABP3* were higher in the mammary tissue and *FABP4* and *ELOVL6* in AT. The consumption of SO did not significantly affect the mRNA abundance of analysed genes in the mammary tissue but caused a decrease of *GPAM* and *SREBF1* in both AT (P<0.05) and of *PPARG* in the subcutaneous depot (interaction diet×AT, P<0.05). There were also differences between AT, with *SREBF1* being more abundant in the subcutaneous (P<0.01) and *LPL* (P<0.05) and *CD36* (P<0.10) in the perirenal AT. The mRNA abundance of *MFGE8* (P<0.001) and *FABP3* (P<0.05) also varied between depots but their levels were always very low.

Discussion

Animal performance and milk fatty acid composition and secretion

The present results provide further support that feeding a moderate amount (2.5% DM) of SO modulates milk FA composition in the absence of negative effects on ewe performance (Toral *et al*., 2010; Gomez-Cortés *et al*., 2011). The observed variations in milk FA composition and secretion (lower levels of medium-chain saturated FA, and greater of c9,t11-CLA and t11-18:1) are related to potentially beneficial effects for human health (Shingfield *et al.*, 2008). Nevertheless, and in contrast to what was expected from previous experiments in ewes fed similar diets (e.g., Toral *et al.*, 2010; Gómez-Cortés *et al.*, 2011), the addition of SO increased the content of t10-18:1,

together with that of t9- and t12-18:1, which might be potentially detrimental to consumers (Shingfield *et al*., 2008). Their concentrations, however, were kept relatively low (≤0.48, 0.53 and 0.81% of total FA, respectively).

Mammary lipid metabolism

The tendency to a reduction in the secretion of <16-carbon FA and the lower milk percentages of <16 and 16-carbon FA in response to SO was not associated with changes in the mRNA abundance in the mammary secretory tissue of the candidate genes involved in *de novo* synthesis (i.e., *ACACA* and *FASN*). These results are consistent with data on gene expression and enzymatic activities reported in goats (Bernard *et al*., 2005b and 2008) and beef cows (Murrieta *et al*., 2006) fed high forage diets supplemented with plant oils and showing no reductions in milk fat synthesis.

The SO treatment also increased the proportion and secretion of milk long-chain FA with no evidences of significant changes in the mRNA abundances of genes related with the uptake, transport and trafficking of long-chain FA in the mammary epithelial cells (i.e., *LPL*, *CD36* and *FABP3*). These findings are consistent with previous research in dairy goats fed plant oils (Bernard *et al.*, 2009a and 2009b; Ollier *et al*., 2009), suggesting that, in the absence of MFD, diet-induced changes in the content of milk long-chain FA might not be mediated by changes in the mRNA abundance of these particular candidate genes. They would also suggest that other regulation mechanisms, such as post-transcriptional events linked to higher availability of 18-carbon FA, may be involved (Bernard *et al.*, 2008). Nonetheless, given the high inter-individual variation in gene expression and the low statistical power of the experiment, it cannot be ruled out that changes are also mediated by modifications in the candidate genes occurring below the level of detection.

With regard to Δ9-desaturation, despite SO modified the milk content of several substrates for (14:0, 16:0, 18:0 and t11-18:1) and products of (c9-18:1 and c9,t11-CLA) mammary SCD, no variation in the milk Δ9-desaturase indexes (used as a proxy for the SCD activity) was observed, indicating that the SO treatment did not affect the activity of this enzyme. In the same way, no changes in the mRNA abundance of *SCD1* and *SCD5* were detected, suggesting that variations in the availability of substrates did not have a significant effect on the transcription of these genes.

This weak relationship between mammary *SCD* mRNA abundance and Δ9-desaturase activity suggests a greater relevance of post-transcriptional regulatory events, such as mRNA stability, initiation of translation or turnover and activity regulation of the enzymatic protein, as observed in rodents (Ntambi, 1999) and ruminants (Bernard *et al.*, 2013).

Concerning the FA profile of the mammary tissue, there is very little literature reporting this data in ruminants (Christie, 1981; Toral *et al.*, 2013) and no information is available in lactating ewes. In the present study, the virtual absence of shorter-chain FA (4 to 9 carbons, which are present in the milk) would suggest not only that residual milk was well removed but also a minor contribution of unsecreted cytosolic lipid droplets to extracted lipids. Nevertheless, the few changes detected in the longer-chain FA in response to SO treatment suggest that variations in milk FA profile and secretion had no significant reflect in mammary FA composition, as previously observed in goats (Toral et al., 2013).

Adipose tissue lipid metabolism

Few changes were observed in lipogenic gene expression in subcutaneous and perirenal AT in response to SO treatment, which agrees with the lack of variation in

energy balance and milk fat secretion, and with data reported in cows and goats (Shingfield *et al.*, 2010). However, the mRNA abundance of *SREBF1* (which might have a central role in lipid synthesis; Harvatine *et al.*, 2009) was decreased in response to SO in both AT (Table 7), and that of *PPARG* in the subcutaneous depot. Although changes in these transcription factors have been related with the up- or down-regulation of several lipogenic genes (Harvatine *et al.,* 2009; Schmitt *et al.*, 2011), no effect of SO on the mRNA abundance of genes related to lipogenesis was detected in the present study, and only *GPAM* mRNA showed a coordinated down-regulation. Yet, since *GPAM* is involved in the esterification of FA to glycerol, this can hardly be related with the few effects of dietary SO on the FA composition of AT (reductions in 18:3n-3 and 17:0), which were probably caused by potential reductions in their availability for tissue uptake, as a result of the negative impact of SO on their ruminal concentration (Toral *et al.*, 2012). Putative effects of SO on the availability of other long-chain FA, as inferred from their changes in milk fat, or in the rumen fluid of ewes fed similar diets (Toral *et al*., 2012) had no substantial consequences on AT composition. Previous data in cows showed that a high availability of exogenous FA (through duodenal infusion of rapeseed oil) had no effect on AT lipogenic activities during early lactation but caused a reduction after the lactation peak, when the activity was observed to increase in cows receiving the control diet due to variation in the energy balance (Chilliard et al, 1991). The few changes observed in the present study in mid-lactation ewes in positive energy balance are likely a result of the relatively low amount of supplemental SO.

When comparing AT, the greater content of saturated FA (mainly 18:0) and *trans*-18:1 isomers (t11- and t12-18:1) in the perirenal AT, and of *cis*-18:1, c9,t11-CLA and n-3 PUFA in the subcutaneous AT, are in line with previous data in ruminants

(Christie, 1981; Bas *et al*., 1987). These specificities due to tissue site have also been correlated with a depot-specific expression of lipogenic genes (Barber *et al.*, 2000; Bernard *et al.*, 2005a). However, the higher proportion of Δ9-desaturase products (mainly c9-14:1, c9-16:1, c9-17:1, c9-18:1 and c9,t11-CLA) and the lower proportion of Δ9-desaturase substrates (mainly 18:0 and t11-18:1) observed in subcutaneous compared to perirenal AT, were not associated with differences in tissue mRNA abundance of *SCD1*, which is consistent with previous research in non-lactating sheep (Barber *et al.*, 2000) but not in goats (Bernard *et al.*, 2005b).

Subcutaneous and perirenal AT exhibited different levels of *LPL* and *CD36* mRNA, suggesting potential divergences in FA uptake. The higher abundance of *LPL* transcripts in the perirenal AT is in line with results of the corresponding enzyme activity in lactating goats (Chilliard *et al*., 1981; Bernard *et al.*, 2005a) and cows (Chilliard and Robelin, 1985). The lack of significant variation due to SO is also consistent with previous data in goats fed soybeans (Bernard *et al.*, 2005a).

Altogether, these results show only marginal changes in the mRNA abundance of the candidate genes and related transcription factors in AT. Although it cannot be dismissed that the lack of more significant differences is due to statistical power limitations of the experiment, they agree with previous observations in goats fed plant lipids (Bernard *et al*., 2005a and 2009a) and in sheep fed marine algae (Bichi *et al*., 2013) for which, respectively, an increase and a decrease of milk fat yield were observed.

Conclusions

This study provides novel and complementary information on the response to diet supplementation with SO in terms of milk FA composition and secretion, and FA

profile and lipogenic gene expression of mammary and adipose tissues in lactating ewes. Dietary inclusion of a moderate amount (2.5% DM) of SO for 7 weeks modifies the FA profile of the milk but has only slight effects on that of mammary and adipose tissues. No relationship is detected between these changes and results on mammary mRNA abundance of the candidate genes and transcription factors involved in lipid metabolism. Neither the marginal repercussion of SO feeding on the gene expression in subcutaneous and perirenal AT, after 7 weeks on the diet, is related with differences in FA profile between adipose sites or with milk fat secretion. Nevertheless, it cannot be ruled out that the response to linoleic-rich diets is mediated by other genes, post-transcriptional mechanisms, or particularly the availability of FA of dietary origin, which would support the need of further studies.

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Table 1 *Ingredients and chemical composition of the experimental diets*[1]

	Diet			
	Control	SO	s.e.d.	P^2
Ingredient (g/kg fresh matter)				
Dehydrated alfalfa hay	600	587	−	−
Whole maize grain	125	123	−	−
Soybean meal solvent 44% CP	100	98	−	−
Whole barley grain	81	80	−	−
Beet pulp, pellets	45	44	−	−
Molasses, liquid	33	32	−	−
Mineral-vitamin mix[3]	16	16	−	−
Sunflower oil[4]	0	22	−	−
Chemical composition (g/kg dry matter)				
Organic matter	901.5	899.3	2.7	ns
CP	153.6	153.9	6.4	ns
NDF	390.0	395.3	37.6	ns
ADF	349.1	350.5	32.8	ns
Ether extract	23.0	48.5	2.1	***
Fatty acid composition (g/100 g fatty acids)				
14:0	0.96	0.42	0.11	***
16:0	21.68	12.15	0.49	***
c9-16:1	0.53	0.40	0.06	†
18:0	4.48	3.81	0.03	***
c9-18:1	10.31	24.26	0.56	***
c11-18:1	0.58	0.71	0.01	***
c9,c12-18:2	29.93	44.85	0.93	***
18:3n-3	17.89	6.96	0.53	***
20:0	0.94	0.52	0.03	***
22:0	1.23	0.90	0.04	***

[1] For each experimental diet, n=7.

[2] Probability of significant effects due to experimental diet. ns=non-significant (P>0.10); †P<0.10; ***P<0.001.

[3] Contained (g/kg): salts [$NaHCO_3$ (458.3), $CaCO_3$ (250.0), NaCl (125.0)], minerals and vitamins (104.2), and wheat bran (62.5).

[4] Carrefour S.A. (Madrid, Spain). Contained (g/100 g fatty acids): 16:0 (5.5), 18:0 (4.4), c9-18:1 (36.4), and c9,c12-18:2 (50.3).

Table 2 *Milk yield and composition in ewes fed a total mixed ration plus 0 (control diet) or 25 g of sunflower oil/kg dry matter (SO diet)*

	Diet			
	Control	SO	s.e.d.	P^1
DM Intake (kg/day)	2.73	2.74	0.10	ns
Energy balance (MJ/day)[2]	5.18	5.48	0.70	ns
Yield (g/day)				
Milk	1280.0	1364.0	116.7	ns
Fat	71.8	75.0	8.1	ns
CP	62.0	67.1	7.2	ns
Lactose	63.2	66.4	6.1	ns
Total solids	209.4	223.3	20.5	ns
Composition (g/100 g)				
Fat	5.60	5.54	0.06	ns
CP	4.82	4.94	0.30	ns
Lactose	4.93	4.87	0.05	ns
Total solids	16.32	16.43	0.66	ns

[1] Probability of significant effects due to experimental diet. ns=non-significant (P>0.10).

[2] Calculated according to INRA (2007).

Table 3 *Fatty acid (FA) composition of milk and mammary tissue from ewes fed a total mixed ration plus 0 (control diet) or 25 g of sunflower oil/kg dry matter (SO diet)*

	Milk				Mammary tissue			
FA (g/100 g FA)	Control	SO	s.e.d.	P^1	Control	SO	s.e.d.	P^1
Saturated FA								
4:0	2.71	2.97	0.19	ns	nd[2]	nd	-	-
6:0	2.38	1.95	0.18	*	nd	nd	-	-
8:0	2.51	1.74	0.18	**	0.06	0.05	0.02	ns
10:0	8.49	4.99	0.58	***	2.61	1.57	1.14	ns
12:0	5.03	3.16	0.35	**	2.70	1.79	0.94	ns
14:0	11.58	9.43	0.61	**	8.16	6.33	1.74	ns
15:0	1.11	0.89	0.07	*	0.97	0.84	0.12	ns
16:0	25.73	21.53	1.18	**	25.90	22.97	1.80	ns
17:0	0.71	0.56	0.06	*	1.10	0.93	0.09	†
anteiso-17:0	0.65	0.51	0.06	*	0.70	0.58	0.06	†
18:0	7.64	11.90	0.78	**	12.01	14.95	1.86	ns
iso-18:0	0.09	0.06	0.01	**	0.12	0.10	0.01	†
Monounsaturated FA								
c9-14:1	0.20	0.18	0.03	ns	0.19	0.16	0.04	ns
c9-16:1	0.78	0.67	0.08	ns	1.01	0.99	0.11	ns
c9-18:1[3]	15.35	21.58	1.13	**	23.67	26.33	2.59	ns
c12-18:1	0.35	0.71	0.05	***	0.61	0.86	0.16	ns
t9-18:1	0.20	0.48	0.03	***	0.31	0.47	0.08	†
t10-18:1	0.26	0.53	0.04	***	0.42	0.49	0.10	ns
t11-18:1	1.32	2.36	0.42	*	1.73	2.46	0.51	ns
t12-18:1	0.32	0.81	0.06	***	0.47	0.71	0.15	ns
Polyunsaturated FA								
c9,c12-18:2	2.81	2.79	0.34	ns	4.40	4.34	0.55	ns
c9,t12-18:2	0.11	0.20	0.01	***	0.11	0.15	0.03	ns
t9,c12-18:2	0.03	0.09	0.01	***	0.05	0.08	0.02	†
t11,c15-18:2	0.13	0.10	0.01	*	0.11	0.10	0.01	ns
c9,t11-CLA[4]	0.69	1.18	0.15	*	0.94	1.30	0.31	ns
Total CLA	0.80	1.30	0.16	*	1.06	1.41	0.32	ns
18:3n-3	1.06	0.75	0.10	*	1.08	0.82	0.13	†
Summary								
Saturated FA	71.80	62.43	2.03	**	57.11	52.63	4.49	ns
Odd- and branched-chain FA	5.64	4.48	0.38	*	5.17	4.47	0.51	ns
Monounsaturated FA	21.96	30.88	1.51	***	33.45	37.67	3.43	ns
PUFA n-3	1.58	1.17	0.13	**	2.15	1.84	0.25	ns
PUFA n-6	3.32	3.38	0.36	ns	6.57	6.71	0.97	ns
<16-carbon FA	36.19	26.92	1.52	***	16.08	11.89	4.14	ns
16-carbon FA	27.52	23.12	1.10	**	27.46	24.50	1.81	ns
>16-carbon FA	36.29	49.97	2.43	**	56.46	63.62	5.64	ns

[1] Probability of significant effects due to experimental diet. ns=non-significant (P>0.10); †P<0.10; *P<0.05; **P<0.01; ***P<0.001.

[2] Non-determined.

[3] In milk, contains c10-, t13-, t14-, and t15-18:1 as minor components. In mammary tissue, contains t13-, and t14-18:1 as minor components.

[4] In milk, contains t7,c9-CLA and t8,c10-CLA as minor components.

Table 4 *Milk fatty acid (FA) yield in ewes fed a total mixed ration plus 0 (control diet) or 25 g of sunflower oil/kg dry matter (SO diet)*

	Milk			
FA yield (g/day)	Control	SO	s.e.d.	P^1
4:0+6:0+8:0	5.02	4.71	0.61	ns
10:0+12:0+14:0	16.86	12.34	2.03	†
16:0	17.21	15.09	1.95	ns
18:0	5.13	8.35	0.96	*
c9-18:1[2]	10.32	15.07	1.46	*
t10-18:1	0.18	0.37	0.04	**
t11-18:1	0.88	1.62	0.25	*
c9,c12-18:2	1.86	1.95	0.25	ns
c9,t11-CLA[3]	0.46	0.81	0.09	**
Total CLA	0.54	0.89	0.10	**
18:3n-3	0.70	0.52	0.07	*
Saturated FA	48.09	43.82	5.57	ns
Odd- and branched-chain FA	3.81	3.13	0.51	ns
Monounsaturated FA	14.77	21.52	1.94	**
PUFA n-3	1.04	0.82	0.10	†
PUFA n-6	2.19	2.36	0.28	ns
<16-carbon FA	24.25	18.92	2.80	†
16-carbon FA	18.42	16.20	2.09	ns
>16-carbon FA	24.34	34.88	3.17	*
c9-14:1/(14:0+c9-14:1)	0.02	0.02	<0.01	ns
c9-16:1/(16:0+c9-16:1)	0.03	0.03	<0.01	ns
c9,t11-CLA/(t11-18:1+c9,t11-CLA)	0.35	0.34	0.02	ns

[1] Probability of significant effects due to experimental diet. ns=non-significant (P>0.10); †P<0.10; *P<0.05; **P<0.01.

[2] Contains c10-, t13-, t14-, and t15-18:1 as minor components.

[3] Contains t7,c9-CLA and t8,c10-CLA as minor components.

Table 5 *Fatty acid (FA) composition of subcutaneous and perirenal adipose tissues (AT) from ewes fed a total mixed ration plus 0 (control diet) or 25 g of sunflower oil/kg dry matter (SO diet)*

	Subcutaneous AT		Perirenal AT			P^1		
FA (g/100 g FA)	Control	SO	Control	SO	s.e.d.	D	T	DxT
Saturated FA								
14:0	2.20	2.50	2.38	2.45	0.23	ns	ns	ns
15:0	0.63	0.57	0.45	0.41	0.05	ns	***	ns
16:0	20.09	21.09	21.45	21.27	1.05	ns	ns	ns
iso-16:0	0.17^{b}	0.20^{ab}	0.21^{a}	0.21^{a}	0.02	ns	**	*
17:0	2.50	2.03	2.09	1.80	0.21	†	**	ns
anteiso-17:0	1.03	0.95	0.82	0.77	0.05	ns	***	ns
18:0	16.82	19.02	34.36	35.72	2.55	ns	***	ns
iso-18:0	0.26	0.25	0.23	0.21	0.02	ns	**	ns
Monounsaturated FA								
c9-14:1	0.09	0.08	0.02	0.03	0.01	ns	***	ns
c9-16:1	1.15	1.07	0.47	0.48	0.15	ns	***	ns
c9-17:1	1.33	0.88	0.39	0.33	0.22	ns	***	ns
c9-18:1	35.68	34.24	22.15	22.46	2.05	ns	***	ns
c10-18:1[2]	2.62	2.58	1.46	1.75	0.23	ns	***	ns
c11-18:1	1.54	1.30	0.91	0.83	0.14	ns	***	ns
t10-18:1	0.51	0.55	0.50	0.43	0.19	ns	ns	ns
t11-18:1	1.78	1.95	2.54	2.27	0.27	ns	**	ns
t12-18:1	0.35	0.39	0.49	0.47	0.05	ns	**	ns
Polyunsaturated FA								
c9,c12-18:2	1.88	1.90	2.02	1.85	0.15	ns	ns	ns
c9,t11-CLA	0.64	0.57	0.35	0.31	0.08	ns	***	ns
Total-CLA	0.73	0.64	0.44	0.37	0.09	ns	***	ns
18:3n-3	0.83	0.70	0.83	0.59	0.08	*	†	ns
t9,t12,c15-18:3	0.012^{b}	0.012^{bc}	0.014^{a}	0.010^{c}	0.001	†	ns	*
c5,c8,c11,c14-20:4	0.06	0.07	0.04	0.03	0.01	ns	***	ns
c8,c11,c14,c17-20:4	0.01	0.02	0.01	0.01	<0.01	ns	**	ns
c7,c10,c13,c16,c19-22:5	0.09	0.10	0.05	0.04	0.02	ns	***	ns
Summary								
Saturated FA	44.93	47.98	63.44	64.39	2.72	ns	***	ns
Odd- and branched-chain FA	5.22	4.69	4.63	4.21	0.32	ns	**	ns
Monounsaturated FA	49.56	46.99	31.95	31.70	2.49	ns	***	ns
PUFA n-3	1.65	1.43	1.39	1.03	0.14	†	***	ns
PUFA n-6	2.09	2.13	2.21	1.99	0.16	ns	ns	†
<16-carbon FA	4.19	4.33	3.47	3.64	0.31	ns	***	ns
16-carbon FA	22.93	23.50	22.85	22.58	1.06	ns	ns	ns
>16-carbon FA	72.28	71.63	72.59	72.77	1.32	ns	ns	ns

[1] Probability of significant effects due to experimental diet (D), tissue (T), and their interaction (DxT). ns=non-significant (P>0.10); †P<0.10; *P<0.05; **P<0.01; ***P<0.001.

[2] Contains t15-18:1 as a minor component.

[a,b,c] Means within a row with different superscripts differ significantly (P<0.05).

Table 6 *mRNA relative abundance (log transformed data) of genes of the mammary tissue involved in lipid metabolism in ewes fed a total mixed ration plus 0 (control diet) or 25 g of sunflower oil/kg dry matter (SO diet).*

(mRNA levels are expressed in arbitrary units determined as the abundance relative to the geometric mean of PPIA, UXT and EIF3K mRNA)

	Mammary tissue			
	Control	SO	s.e.d.	P^1
Lipogenic genes				
ACACA	1.31	1.32	0.08	ns
FASN	1.88	1.79	0.10	ns
LPL	1.75	1.66	0.08	ns
CD36	1.86	1.92	0.06	ns
FABP3	1.63	1.59	0.14	ns
FABP4	0.54	0.55	0.06	ns
SCD1	1.63	1.63	0.08	ns
SCD5	1.73	1.78	0.08	ns
GPAM	1.72	1.83	0.08	ns
CPT2	1.62	1.66	0.03	ns
ELOVL5	0.90	0.83	0.14	ns
ELOVL6	-0.10	-0.28	0.13	ns
MFGE8	1.72	1.73	0.11	ns
XDH	1.81	1.82	0.08	ns
Transcription factors				
SREBF1	1.79	1.76	0.10	ns
PPARG	0.95	0.95	0.05	ns

[1] Probability of significant effects due to experimental diet. ns=non-significant (P>0.10).

Table 7 *mRNA relative abundance (log transformed data) of genes of subcutaneous and perirenal adipose tissues (AT) involved in lipid metabolism in ewes fed a total mixed ration plus 0 (control diet) or 25 g of sunflower oil/kg dry matter (SO diet).*

(mRNA levels are expressed in arbitrary units determined as the abundance relative to the geometric mean of PPIA, UXT and EIF3K mRNA)

	Subcutaneous AT		Perirenal AT			P^1		
	Control	SO	Control	SO	s.e.d.	D	T	DxT
Lipogenic genes								
ACACA	1.82	1.58	1.60	1.58	0.18	ns	ns	ns
FASN	1.38	1.14	1.19	1.14	0.25	ns	ns	ns
LPL	1.70	1.66	1.83	1.82	0.12	ns	*	ns
CD36	1.82	1.81	1.88	1.90	0.06	ns	†	ns
FABP3	-2.84	-2.61	-3.08	-2.94	0.09	ns	*	ns
FABP4	1.87	1.83	1.87	1.91	0.08	ns	ns	ns
SCD1	1.69	1.71	1.67	1.56	0.16	ns	ns	ns
SCD5	1.00	1.02	1.19	1.17	0.17	ns	ns	ns
GPAM	1.42	1.09	1.39	1.28	0.12	*	ns	ns
CPT2	1.89	1.83	1.83	1.82	0.08	ns	ns	ns
ELOVL5	1.62	1.73	1.68	1.78	0.13	ns	ns	ns
ELOVL6	1.79	1.61	1.77	1.74	0.17	ns	ns	ns
MFGE8	0.202	0.231	-0.002	-0.052	0.109	ns	***	ns
XDH	-2.74	-2.83	-2.82	-2.82	0.20	ns	ns	ns
Transcription factors								
SREBF1	1.60	1.40	1.39	1.10	0.11	*	**	ns
PPARG	1.92^{a}	1.77^{b}	1.87^{ab}	1.86^{a}	0.06	ns	ns	*

[1] Probability of significant effects due to experimental diet (D), tissue (T), and their interaction (DxT). ns=non-significant (P>0.10); †P<0.10; *P<0.05; **P<0.01; ***P<0.001.

[a,b] Means within a row with different superscripts differ significantly (P<0.05).

SUPPLEMENTARY FILE (for online publication only)

Supplementary Table S1 *Primer and probe sequences and conditions used for real-time reverse transcription-PCR*

Gene	Encoded protein	Accession no.	Nucleotide sequence (5' → 3')[1]	T[2]	Source
ACACA	Acetyl-CoA carboxylase alpha	NM_001009256	F: CAT GGA AAT GTA CGCGGA CC R: GGT GGT AGA TGG GAA GGA GG P: CGA GCG GAA GGA GCT GGA GAG CA	58	Bernard *et al*., 2005
FASN	Fatty acid synthase	DQ223929	F: ACA GCC TCT TCC TGT TTG ACG R: CTC TGC ACG ATC AGC TCG AC P: ATC TGG AGG CGC GTG TGG CAG CC	60	Bernard *et al*., 2005
LPL	Lipoprotein lipase	AF228667	F: TTC AGA GGC TAT TAC TGG AAA TCC R: ATG TCA ATC ACA GCA TTC ATT CTA CT P: TTC CAG TGG TGC CGG AAC ACT CCT TC	60	Bernard *et al*., 2005
CD36	Thombospondin receptor	X91503	F: ACA GAT GTG GCT TGA GCG TG R: ACT GGG TCT GTG TTT TGC AGG	58	Bernard *et al*., 2012
FABP3	Fatty acid-binding protein 3, heart	BT021486	F: CCT CTC CTT CCA CTG ACT GC R: TTG ACC TCA GAG CAC CCT TT	58	Jurie *et al*., 2007
FABP4	Fatty acid-binding protein 4, adipocyte	NM_174314	F: GGT ACC TGG AAA CTT GTC TCC R: CTG ATT TAA TGG TGA CCA CAC	58	Jurie *et al*., 2007
SCD1	Stearoyl-CoA desaturase 1	AF325499	F: TGC TGA CAA CTT ATC TGG ATG C R: AAG GAA TCC TGC AAA CAG CTA P: CCA GAG CCT GCA GAA GTG GCT GGT ATA A	60	Bernard *et al*., 2005
SCD5	Stearoyl-CoA desaturase 5	NM_001112815	F: AGA AGG GGA GGA AGC TTG AC R: GGA GGC CAG GAA GTA GGA GT	58	Lengi and Corl, 2007
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	NM_001012282	F: ACC AGC AGT TCA TCA CCT TC R: GTA CAC GGC AAC CCT CCT CT	58	Faulconnier *et al*., 2011
CPT2	Carnitine palmitoyl transferase 1B	NM_001045889	F: GCC TCT GTT TCA GCA TAA R: GGT GCT CAG GCA CCT CAT A	60	This article
ELOVL5	Elongation of very long chain fatty acids like 5	NM_001046597	F: CTG AAT ACC TTC TCC ACT GGA GGA R: GCT CCC TGT AAT ATG AAT GTG CAA	60	Faulconnier et al., 2011
ELOVL6	Elongation of very long chain fatty acids like 6	NP_001095625	F: CAA TAT TTT CCC AGG GTT R: AGC TGC CCT TTC AAG AGT TG	62	This article
MFGE8	Milk fat globule-EGF factor 8	NM_005928	F: TGA GTA GGT CTG GGA TGG AC R: GGA AGC TGC CTG TGT ACT CT	60	Ollier *et al*., 2007
XDH	Xanthine dehydrogenase oxidase	X83508	F: GCC CTG CAG AAC ATG AAT CT R: GCA CAA ATA CTT CCT ACA CCT	60	Ollier *et al*., 2009

SREBF1	Sterol regulatory element binding transcription factor 1	TC263657	F: CCA GCT GAC AGC TCC ATT GA R: TGC GCG CCA CAA GGA	60	Harvatine and Bauman, 2006
PPARG	Peroxisome proliferator-activated receptor gamma	NM_177945	F: CAG GTT TGA AAG AAG CCA CA R: TTA CGG AAA CGT CCC TCT TG	60	Bonnet *et al*., 2007
PPIA	Cyclophilin A	XM_001252497	F: GGA TTT ATG TGT CCA GGG TGG TGA R: CAA GAT GCC AGG ACC TGT ATG P: TCT CCC CAT AGA TGG ACT TGC CAC CAG	60	Bonnet *et a*l., 2000
UXT	Ubiquitously-expressed transcript	BQ676558	F: TGT GGC CCT TGG ATA TGG TT R: GGT TGT CGC TGA GCT CTG TG	60	Kadegowda *et al*., 2009
EIF3K	Eukaryotic translation initiation factor 3 subunit K	NM_001034489	F: CCA GGC CCA CCA AGA AGA A R: TTA TAC CTT CCA GGA GGT CCA TGT	60	Kadegowda *et al*.,2009

[1] Sequences: F = forward primer; R = reverse primer; P = Taqman probe.

[2] T = PCR annealing temperature (°C).

Supplementary Table S2 *Other fatty acids (FA) of milk from ewes fed a total mixed ration plus 0 (control diet) or 25 g of sunflower oil/kg dry matter (SO diet).*

(to complete the FA profile shown in Table 3)

	Milk			
FA (g/100 g FA)	Control	SO	s.e.d.	*P* [1]
5:0	0.02	0.01	<0.01	ns
7:0	0.03	0.02	<0.01	**
9:0	0.06	0.03	0.01	**
c9-10:1	0.30	0.18	0.02	**
11:0	0.09	0.04	0.01	**
c9-12:1	0.09	0.04	0.01	**
t9-12:1	0.05	0.03	<0.01	**
iso-13:0	0.03	0.02	<0.01	ns
anteiso-13:0	0.010	0.007	0.001	*
4,8,12-trimethyl-13:0	0.24	0.23	0.01	ns
iso-14:0	0.14	0.10	0.02	*
c7-14:1	0.01	0.01	<0.01	ns
c12-14:1	0.09	0.04	0.01	**
t5-14:1	0.02	0.02	<0.01	ns
iso-15:0	0.28	0.26	0.02	ns
anteiso-15:0	0.52	0.42	0.05	*
t5-15:1	0.18	0.13	0.02	*
t6-,7-15:1	0.03	0.02	<0.01	*
iso-16:0	0.04	0.04	<0.01	ns
3,7,11,15-tetramethyl-16:0	0.35	0.27	0.045	ns
c7-16:1	0.29	0.32	0.03	ns
c14-16:1	0.16	0.07	0.02	**
t5-16:1	0.03	0.02	<0.01	*
t6-,8-16:1	0.08	0.10	0.01	ns
t9-16:1	0.09	0.14	0.02	*
iso-17:0	0.40	0.34	0.04	ns
c9-17:1	0.27	0.20	0.02	*
t7-17:1	0.02	0.02	0.01	ns
10-oxo-18:0	0.03	0.03	0.01	ns
13-oxo-18:0	0.03	0.02	<0.01	**
c11-18:1	0.55	0.53	0.04	ns
c13-18:1	0.07	0.11	0.02	*
c15-18:1	0.09	0.17	0.01	***
c16-18:1	0.08	0.14	0.01	***
t4-18:1	0.02	0.04	<0.01	**
t5-18:1	0.02	0.04	<0.01	**
t6-,7-,8-18:1	0.19	0.43	0.03	**
t16-18:1[2]	0.35	0.66	0.05	***
c9,c15-18:2	0.15	0.10	0.01	**
c9,t13-18:2	0.24	0.50	0.05	**
9,14-18:2	0.07	0.15	0.01	**

t9,t12-18:2	0.006	0.012	0.002	**
t9,c11-CLA	0.02	0.03	<0.01	*
t10,c12-CLA	0.005	0.007	0.001	*
t11,t13-CLA	0.01	0.01	<0.01	ns
other trans,trans-CLA[3]	0.08	0.07	0.01	*
c6,c9,c12-18:3	0.09	0.04	0.01	**
c9,t11,c15-18:3	0.02	0.02	0.01	ns
t9,t12,c15-18:3	0.02	0.01	<0.01	***
t9,t12,t15-18:3	0.04	0.04	0.01	ns
19:0[4]	0.12	0.11	0.01	ns
20:0	0.26	0.29	0.01	*
c5-20:1	0.08	0.09	0.01	ns
c11-20:1	0.07	0.07	0.01	ns
c11,c14-20:2	0.03	0.02	<0.01	*
c11,c14,c17-20:3	0.03	0.03	0.01	ns
c5,c8,c11,c14-20:4	0.20	0.18	0.02	ns
c8,c11,c14,c17-20:4	0.02	0.01	<0.01	*
c5,c8,c11,c14,c17-20:5	0.06	0.05	<0.01	***
21:0	0.11	0.09	0.01	*
22:0	0.21	0.25	0.02	ns
c7,c10,c13,c16-22:4	0.04	0.03	0.01	ns
c7,c10,c13,c16,c19-22:5	0.15	0.12	0.02	ns
c4,c7,c10,c13,c16,c19-22:6	0.05	0.05	0.01	ns
23:0	0.15	0.10	0.02	*
24:0	0.07	0.06	<0.01	ns
c15-24:1	0.03	0.03	0.01	ns

[1] Probability of significant effects due to experimental diet. ns=non-significant ($P>0.10$); *$P<0.05$; **$P<0.01$; ***$P<0.001$.

[2] Coelutes with 10,14-18:2.

[3] Sum of t7,t9-CLA + t8,t10-CLA + t9,t11-CLA + t10,t12-CLA.

[4] Contains t11,t15-18:2 as a minor component.

Supplementary Table S3 *Other fatty acids (FA) of mammary tissue from ewes fed a total mixed ration plus 0 (control diet) or 25 g of sunflower oil/kg dry matter (SO diet).*

(to complete the FA profile shown in Table 3)

	Mammary tissue			
FA (g/100 g FA)	Control	SO	s.e.d.	*P* [1]
c9-12:1	0.06	0.04	0.03	ns
iso-15:0	0.19	0.21	0.04	ns
anteiso-15:0	0.42	0.35	0.08	ns
t9-14:1[2]	0.06	0.01	0.04	ns
iso-16:0	0.33	0.29	0.06	ns
t9-16:1	0.08	0.11	0.03	ns
iso-17:0[3]	0.72	0.70	0.04	ns
c9-17:1	0.48	0.39	0.05	ns
c10-18:1[4]	1.55	1.62	0.16	ns
c11-18:1	1.14	1.13	0.11	ns
c13-18:1	0.13	0.14	0.02	ns
c15-18:1	0.15	0.17	0.02	ns
c16-18:1	0.06	0.07	0.01	ns
t4-18:1	0.03	0.04	0.01	ns
t5-18:1	0.01	0.02	0.01	†
t6-,7-,8-18:1	0.21	0.34	0.07	†
t16-18:1	0.34	0.42	0.06	ns
c9,c15-18:2	0.24	0.21	0.02	ns
c9,t13-18:2	0.36	0.43	0.08	ns
9,14-18:2	0.16	0.20	0.03	ns
10,14-18:2	0.12	0.16	0.03	ns
t9,c11-CLA	0.05	0.06	0.01	ns
t10,c12-CLA	0.02	0.02	0.01	ns
t11,t13-CLA	0.05	0.03	0.01	†
other *trans*,*trans*-CLA[5]	0.05	0.06	0.01	ns
c6,c9,c12-18:3	0.03	0.02	<0.01	†
19:0[6]	0.12	0.11	0.01	ns
20:0	0.29	0.28	0.03	ns
c5-20:1	0.02	0.03	<0.01	ns
c11-20:1	0.14	0.14	0.02	ns
c11,c14-20:2	0.09	0.07	0.03	ns
c8,c11,c14-20:3	0.08	0.09	0.02	ns
c11,c14,c17-20:3	0.03	0.02	0.02	ns
c5,c8,c11,c14-20:4	0.57	0.61	0.13	ns
c5,c8,c11,c14,c17-20:5	0.13	0.13	0.01	ns
21:0	0.09	0.07	0.02	ns
22:0	0.15	0.15	0.02	ns
c7,c10,c13,c16-22:4	0.11	0.11	0.13	ns
c7,c10,c13,c16,c19-22:5	0.44	0.43	0.01	ns
c4,c7,c10,c13,c16,c19-22:6	0.13	0.13	0.03	ns

23:0	0.09	0.07	0.02	ns

[1] Probability of significant effects due to experimental diet. ns=non-significant (P>0.10); † P<0.10.

[2] Coelutes with iso-15:0.

[3] Coelutes with c7-16:1

[4] Contains t15-18:1 as minor component.

[5] Sum of t7,t9-CLA + t8,t10-CLA + t9,t11-CLA + t10,t12-CLA.

[6] Contains t11,t15-18:2 as minor component.

Supplementary Table S4 *Other fatty acids (FA) of subcutaneous and perirenal adipose tissues (AT) from ewes fed a total mixed ration plus 0 (control diet) or 25 g of sunflower oil/kg dry matter (SO diet).*

(to complete the FA profile shown in Table 5)

	Subcutaneous AT		Perirenal AT			*P* [1]		
FA (g/100 g FA)	Control	SO	Control	SO	s.e.d.	D	T	DxT
10:0	0.06	0.09	0.08	0.11	0.01	*	*	ns
12:0	0.06	0.07	0.05	0.06	0.01	ns	**	ns
t9-14:1	0.05	0.03	0.01	0.01	0.01	ns	**	ns
c13-18:1	0.15	0.13	0.10	0.08	0.01	†	***	ns
iso-15:0	0.12	0.15	0.15	0.17	0.02	ns	***	†
anteiso-15:0	0.17	0.18	0.20	0.20	0.02	ns	*	ns
3,7,11,15-tetramethyl-16:0	0.03	0.04	0.05	0.04	<0.01	ns	***	†
t9-16:1	0.04	0.03	0.03	0.03	<0.01	*	**	ns
iso-17:0	0.37	0.38	0.37	0.35	0.02	ns	†	ns
iso-18:0	0.26	0.25	0.23	0.21	0.02	ns	**	ns
c12-18:1	0.53	0.54	0.49	0.47	0.04	ns	*	ns
c15-18:1	0.19	0.19	0.18	0.16	0.02	ns	*	ns
c16-18:1	0.08	0.08	0.09	0.08	0.01	ns	*	ns
t4-18:1	0.01	0.01	0.01	0.01	<0.01	ns	ns	ns
t5-18:1	0.01	0.01	0.01	0.01	<0.01	ns	ns	ns
t6-,7-,8-18:1	0.26	0.23	0.32	0.25	0.05	ns	ns	ns
t9-18:1	0.25	0.25	0.25	0.25	0.02	ns	ns	ns
t16-18:1	0.29	0.30	0.45	0.37	0.09	ns	ns	ns
c9,c15-18:2	0.36	0.33	0.21	0.18	0.03	ns	***	ns
c9,t12-18:2	0.01	0.03	0.04	<0.01	0.02	ns	ns	†
c9,t13-18:2	0.38	0.31	0.20	0.16	0.04	ns	***	ns
9,14-18:2	0.16	0.13	0.03	0.07	0.03	ns	***	†
10,14-18:2	0.10	0.09	0.10	0.08	0.01	ns	ns	ns
t9,c12-18:2	0.03	0.03	0.03	0.03	<0.01	ns	ns	ns
t11,c15-18:2	0.27	0.23	0.25	0.18	0.04	ns	†	ns
t9,c11-CLA	0.04	0.03	0.02	0.02	0.01	ns	**	ns
t10,c12-CLA	0.011	0.011	0.013	0.012	0.011	ns	†	ns
t11,t13-CLA	0.03	0.03	0.06	0.04	0.01	ns	*	ns
other *trans*,*trans*-CLA[2]	0.04	0.05	0.04	0.04	<0.01	ns	ns	ns
c6,c9,c12-18:3	0.02	0.02	0.02	0.02	<0.01	ns	ns	ns
19:0[3]	0.21	0.22	0.31	0.28	0.02	ns	***	ns
20:0	0.08	0.10	0.16	0.18	0.01	ns	***	ns
c11-20:1	0.14	0.15	0.15	0.13	0.03	ns	ns	ns
c13-22:1	0.01	0.01	0.01	0.01	0.01	ns	ns	ns
c11,c14-20:2	0.03	0.02	0.02	0.02	0.01	ns	ns	ns
c8,c11,c14-20:3	0.02	0.02	0.01	0.01	<0.01	ns	*	ns
c11,c14,c17-20:3	0.02	0.02	0.01	0.01	<0.01	ns	*	ns
c8,c11,c14,c17-20:4	0.01	0.02	0.01	0.01	<0.01	ns	**	ns
21:0	0.008[c]	0.01	0.021[a]	0.021[a]	0.010	ns	***	*
22:0	0.01	0.01	0.02	0.02	<0.01	ns	*	ns

c7,c10,c13,c16-22:4	0.014	0.014	0.008	0.007	0.005	ns	**	ns
c4,c7,c10,c13,c16,c19-22:6	0.02	0.02	0.01	<0.01	0.01	ns	*	ns
23:0	0.01	0.02	0.02	0.02	<0.01	ns	ns	ns

[1] Probability of significant effects due to experimental diet (D), tissue (T), and their interaction (DxT). ns=non-significant (P>0.10); † P<0.10; *P<0.05; **P<0.01; ***P<0.001.

[2] Sum of t7,t9-CLA + t8,t10-CLA + t9,t11-CLA + t10,t12-CLA.

[3] Contains t11,t15-18:2 as minor component.

[a,b,c] Means within a row with different differ significantly (P<0.05).

Supplementary Table S5 *Real-time reverse transcription -PCR performance of lipogenic genes, transcription factors and internal controls.*

	Ct[1]			R^2 statistic[2]	Efficiency[3]
	Mammary tissue	Subcutaneous AT	Perirenal AT		
Lipogenic genes					
ACACA	32-33	27-32	28-32	0.974	1.85
FASN	27-29	26-32	27-31	0.999	1.80
LPL	26-28	23-29	23-27	0.998	1.54
CD36	18-19	17-18	16-17	0.998	1.60
FABP3	15-17	25-28	26-28	0.913	1.91
FABP4	27-28	21-23	21-23	0.912	1.90
SCD1	25-26	22-24	21-25	0.997	1.73
SCD5	31-33	31-35	31-33	0.975	2.10
GPAM	20-22	19-22	19-22	0.996	1.76
CPT2	30-31	26-28	26-29	0.995	1.78
ELOVL5	31-33	26-30	26-29	0.984	1.92
ELOVL6	32-35	23-27	23-27	0.999	1.72
MFGE8	21-23	25-28	26-28	0.998	1.60
XDH	19-20	34-35	33-36	0.965	1.81
Transcription factors					
SREBF1	28-29	27-29	27-30	0.996	2.02
PPARG	28-29	23-25	23-25	0.993	1.85
Internal controls					
PPIA	26-27	24-26	24-26	0.979	1.83
UXT	27-28	26-27	25-27	0.998	1.94
EIF3K	25-27	23-26	22-25	0.997	1.74

[1]Cycle threshold.

[2]Coefficient of determination (R^2) of the standard curve.

[3]The efficiency was calculated as [$10^{(-1 / Slope)}$].

Supplementary Material S1 *References used in Supplementary Table S1.*

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