Reductions in milk Δ^9 -desaturation ratios to oral dosing of cobalt-acetate are accompanied by the downregulation of *SCD1* in lactating ewes

P. G. Toral,¹ G. Hervás, and P. Frutos

Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n, 24346 Grulleros, León, Spain

ABSTRACT

Oral administration of cobalt has been proven to alter milk fatty acid (FA) composition consistent with an inhibition of mammary stearoyl-coenzyme A desaturase (SCD) activity in ruminants, but the mechanisms explaining its mode of action remain uncertain. In this study, Co (as Co-acetate) was dosed to lactating ewes with the aims of examining mammary gene expression during Co-induced changes in milk FA composition, and estimating the endogenous synthesis of SCD products in milk of sheep fed an 18:3n-3-enriched diet. Twelve Assaf ewes fed a diet supplemented with 2% linseed oil were allocated to 2 experimental groups and received an oral drench supplying either 0 (control) or 9 mg of Co/kg of body weight per day. Treatments were administered in 3 equal doses at 8-h intervals for 6 d. No effects of Co administration on animal performance were observed. The changes in milk FA (namely, reductions in most *cis*-9-containing FA) were consistent with an inhibition of SCD in the absence of detectable effects on the relative importance of mammary de novo synthesis and FA uptake. The high proportion of endogenous *cis*-9 *trans*-11 18:2 observed in this study (89%) would agree with a greater supply of *trans*-11 18:1 of ruminal origin in ewes fed linseed oil, compared with previous estimates in sheep fed a diet without lipid supplementation. Differences between studies could also be related to diet-induced changes in SCD activity. Altogether, both mechanisms would support that basal diet composition is a major determinant of the relative contribution of Δ^9 -desaturation to milk FA profile. Similarly, the consumption of a diet rich in 18:3n-3 might also explain the low proportion of milk *cis*-9 18:1 estimated to derive from Δ^9 -desaturation (29%). The administration of Co to ewes fed linseed oil allowed to discriminate minor 18:3 isomers in milk, such as *cis*-9 *trans*-12 *cis*-15 18:3, as SCD products. Finally, Co dosing lowered the mRNA abundance of *SCD1* in the mammary secretory tissue (33%), whereas

no changes were detected in the *SCD5* isoform or in the studied transcription factors (*SREBF1*, *PPARG*, *SP1*, and *EGR2*). These results suggest that the mode of action of Co in dairy ewes would be at least partly mediated by the downregulation of *SCD1*.

Key words: gene expression, lipid metabolism, mRNA, sheep, stearoyl-coenzyme A desaturase

INTRODUCTION

Stearoyl-CoA desaturase (SCD) plays a key role in the regulation of milk fat composition and nutritional quality in ruminants. This Δ^9 -desaturase is responsible for introducing a *cis* double bond between carbons 9 and 10 of the FA, which accounts for most of *cis*-9 *trans*-11 CLA and a considerable proportion of *cis*-9 18:1 appearing in milk (Griinari et al., 2000; Mosley and McGuire, 2007). Given the potential benefits of these FA to human health, increasing their content in ruminant-derived products is a desirable target in animal feeding (Lock and Bauman, 2004; Bichi et al., 2013; Shingfield et al., 2013) that would explain the interest in the SCD enzymatic complex (Griinari et al., 2000; Mosley and McGuire, 2007; Bernard et al., 2013).

The administration of cobalt in a chelated form (i.e., as Co-acetate or Co-EDTA) has been proven to alter milk FA composition consistent with an inhibition of Δ^9 -desaturation (Shingfield et al., 2008; Taugbøl et al., 2008; Frutos et al., 2014), therefore representing a cost-effective alternative to other more expensive and involved methods to study SCD activity in lactating ruminants (Griinari et al., 2000; Toral et al., 2012a). As happens with the use of stercularic acid, cobalt decreases *cis*-9-containing FA in milk and allows estimating the proportion that is potentially derived from endogenous synthesis (Griinari et al., 2000; Frutos et al., 2014). Thus, for instance, by means of Co-acetate and stercularic acid administration, Δ^9 -desaturation has been estimated to account for 51 and 74% of *cis*-9 *trans*-11 18:2 found in ewe milk, respectively (Bichi et al., 2012; Frutos et al., 2014); this variation being attributed to differences in diet FA composition (TMR vs. pasture; i.e., supplying mainly 18:2n-6 or 18:3n-3, respectively) rather than in methodological approaches. However,

Received August 11, 2014.

Accepted November 13, 2014.

¹Corresponding author: pablo.toral@csic.es

although the relative proportion of dietary 18:2n-6 vs. 18:3n-3 is known to affect the accumulation of *trans*-11 18:1 in the rumen and the amount of milk *cis*-9 *trans*-11 18:2 of ruminal origin (AbuGhazaleh et al., 2003; Loor et al., 2005), no studies are available on the effect of Δ^9 -desaturase on the endogenous synthesis of FA in dairy ewes fed diets enriched in 18:3n-3.

The underlying mechanisms of cobalt also remain uncertain. Recent investigations suggest that Co would not inhibit mammary SCD at a transcriptional level in cattle (Karlengen et al., 2012; Vilkkii et al., 2012), but the regulation of *SCD* gene expression is complex (Bernard et al., 2013), and no data are available, for example, on the effects on the second *SCD* gene isoform in ruminants (*SCD5*) or the transcription factors that might regulate their expression (e.g., *SREBF1*, *PPARG*, *SP1*, and *EGR2*; Lengi and Corl, 2012; Bernard et al., 2013). In addition, even though the responses to Co observed in ewes differ from those in cows, and indirect comparisons provide further indications of inherent interspecies differences in SCD activity (Shingfield et al., 2008; Frutos et al., 2014), no information is available regarding the effects of Co on mammary gene expression in sheep.

Therefore, we conducted this study in dairy ewes fed a diet supplemented with linseed oil to which we administered Co-acetate with the aims of 1) examining mammary gene expression during Co-induced changes in milk FA composition (consistent with an inhibition of the SCD system), and 2) estimating the contribution of mammary Δ^9 -desaturation to milk concentrations of SCD products in sheep fed an 18:3n-3-enriched diet.

MATERIALS AND METHODS

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 (BW = 78.5 ± 2.43 kg; DIM = 125 ± 1.7) were housed in individual tie stalls and allocated to 1 of 2 groups (n = 6) that were balanced according to milk production and composition, BW, DIM, and parity. All ewes were fed a TMR (forage:concentrate ratio 50:50, DM basis) based on alfalfa hay (particle size > 4 cm) and concentrates and supplemented with 2% DM of linseed oil (Vandeputte Oléochemicals S.A., Mouscron, Belgium). The formulation and chemical composition are shown in Table 1. The TMR was offered twice daily, at 0900 and 1900 h, to ensure ad libitum intakes. Ewes had continuous access to clean drinking water and were milked at approximately 0830 and 1830 h in a dedi-

cated 1 × 10 stall-milking parlor (DeLaval, Madrid, Spain).

The experiment included an 18-d adaptation period before commencing the Co administration to ensure stable effects of the diet on milk FA composition. Then, groups were allocated to 2 experimental treatments and received an oral drench supplying either 0 (control) or 9 mg of Co (**Co9**)/kg of BW per day for 6 d. Cobalt supplied in the form of cobalt(II) acetate tetrahydrate (Sigma-Aldrich, Madrid, Spain) was dissolved in 450 mL of water and administered orally. Sheep were drenched with one-third of the daily dose at 8-h intervals (0800, 1600, and 2400 h). Control animals received only water, following the same pattern.

Measurements and Sampling Procedures

Diets. Samples of the TMR offered and refusals were collected and weighed daily, stored at -30°C , and then freeze-dried before chemical analysis.

Milk. Milk yield was recorded daily. On the last 3 d of the adaptation period, and on d 3, 5, and 6 of Co administration, individual milk samples were collected and composited according to morning and evening milk yield. One aliquot of composite milk was stored at -30°C until milk FA composition analysis, whereas another aliquot was preserved with bronopol (D & F Control Systems Inc., San Ramon, CA) and stored at 4°C until analyzed for fat, CP, lactose, and TS.

Biopsies. At the end of the experimental period, mammary tissue samples were biopsied from each ewe. After local anesthesia of the area by subcutaneous injection of lidocaine (Anesvet, Laboratorios Ovejero S.A., León, Spain), a 4-cm incision was made at the midpoint section of a half-udder, through the skin and underlying fascia until the mammary capsule was clearly visible. Tissue samples (≈ 25 mg) were collected using disposable needles (12 G × 10 cm, MN1210, Bard Magnum, Bard Biopsy Systems, Tempe, AZ) for the Magnum reusable core biopsy instrument (MG1522, Bard Biopsy Systems), immediately frozen in liquid N_2 and stored at -80°C until RNA extraction. The wound was treated with adrenaline (B. Braun S.A., Barcelona, Spain), and the incision was closed with suture clips and sprayed with topical antibiotic (Tenicol Spray, MSD Animal Health, Madrid, Spain) and a wound-healing product (Aluspray, Vétoquinol Especialidades Veterinarias S.A., Madrid, Spain). Ewes also received injections of antibiotic (Eupen Plus, Fatro Uriach Veterinaria, S.L., Barcelona, Spain), antiinflammatory (Ketofen, Merial Laboratorios S.A., Barcelona, Spain), and systemic hemostatic drugs (Hemo 141, Esteve Veterinaria, Barcelona, Spain). No incidences of mastitis were detected for any ewe.

Table 1. Formulation and chemical composition of the experimental diet

Item	TMR ¹	SEM
Ingredient (g/kg of fresh matter)		
Dehydrated alfalfa hay	491	—
Whole corn grain	138	—
Whole barley grain	98	—
Soybean meal solvent 44% CP	147	—
Sugar beet pulp (pellets)	49	—
Molasses (liquid)	39	—
Linseed oil ²	18	—
Mineral supplement ³	18	—
Vitamin supplement ⁴	2	—
Chemical composition (g/kg of DM)		
OM	890	3.2
CP	191	4.7
NDF	289	5.5
Total FA	36	0.6
FA composition (g/100 g of total FA)		
16:0	14.0	0.11
18:0	4.4	0.04
<i>cis</i> -9 18:1	15.3	0.22
18:2n-6	23.2	0.53
18:3n-3	37.0	0.72

¹n = 4.²Raw linseed oil + 500 mg/kg of Oxy-nil (Vandeputte Oléochemicals S.A., Mouscron, Belgium); contained (% total FA): 16:0 (6.2), 18:0 (4.2), *cis*-9 18:1 (17.9), 18:2n-6 (15.6), and 18:3n-3 (54.2).³Declared as containing (g/kg): CaCO₃ (556), Ca₂HPO₄ (222), and NaCl (222).⁴VITAFAC 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), Fe (17.5 g/kg), Mn (20 g/kg), Co (50 mg/kg), I (250 mg/kg), Zn (15 g/kg), Se (100 mg/kg), sepiolite (100 g/kg), Ca (26.2 g/kg), and Mg (6.15 g/kg).

Laboratory Analysis

Diets. Samples were analyzed for DM (ISO 6496:1999), ash (ISO 5984:2002), and CP (ISO 5983–2:2009). Neutral detergent fiber was determined using an Ankom²⁰⁰⁰ fiber analyzer (NDF Method 13; Ankom Technology Corp., Macedon, NY), assayed with sodium sulfite and α -amylase, and expressed with residual ash. Fatty acid methyl esters of lipid in freeze-dried samples of TMR were prepared in a 1-step extraction-transesterification procedure using chloroform (Sukhija and Palmquist, 1988) and 2% (vol/vol) sulfuric acid in methanol (Shingfield et al., 2003), and *cis*-12 tridecenoate (Larodan Fine Chemicals AB, Malmö, Sweden) as an internal standard.

Milk. Fat, CP, lactose, and TS concentrations were determined by infrared spectrophotometry (ISO 9622:1999) using a MilkoScan FT6000 (Foss, Hillerød, Denmark). Lipid in 1 mL of milk was extracted using diethylether and hexane (5:4, vol/vol) and transesterified to FAME using freshly prepared methanolic sodium methoxide as a catalyst (Shingfield et al., 2003). Methyl esters were separated and quantified using a gas chromatograph (Agilent 7890A GC System, Agilent Technologies Inc., Santa Clara, CA) equipped with a flame-ionization detector and a 100-m fused silica capillary column (0.25 mm i.d., 0.2- μ m film thickness; CP-

SIL 88, CP7489, Varian Ibérica S.A., Madrid, Spain) and hydrogen as the carrier gas (207 kPa, 2.1 mL/min). The total FAME profile in a 2- μ L sample volume at a split ratio of 1:50 was determined using a temperature gradient program (Shingfield et al., 2003). Isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170°C (Shingfield et al., 2003). Peaks were identified based on retention time comparisons with commercially available authentic standards (Nu-Chek Prep, Elysian, MN; Sigma-Aldrich, Madrid, Spain; and Larodan Fine Chemicals AB, Malmö, Sweden), cross referencing with chromatograms reported in the literature (Shingfield et al., 2003; Halmemies-Beauchet-Filleau et al., 2011) and comparison with reference 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 (Bichi et al., 2013).

RNA Isolation and Quantitative PCR. Total RNA from the mammary secretory tissue was isolated and purified (PureLink RNA Mini kit, Invitrogen, Carlsbad, CA) as described in Bonnet et al. (2013), with the exception that tissue was homogenized using a bead beater (Mini-bead Beater-8, BioSpec Products, Bartlesville, OK). The concentration and purity of RNA was determined by spectrophotometry using a Nano-Drop ND-1000 spectrophotometer (Nano-Drop

Technologies, Wilmington, DE), and RNA integrity by capillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies Inc.). The average RNA integrity number (RIN) of the samples was 7.9 ± 0.09 . For quantitative PCR (qPCR) analysis, cDNA was synthesized using the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA) and the lack of DNA contamination was verified by PCR amplification using *ACTB* primers flanking an intron (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2014-8731>). The qPCR was carried out (Bonnet et al., 2013) using a 7500 Real-Time PCR System (Applied Biosystems) and specific primers for genes encoding stearoyl-CoA desaturase 1 (*SCD1*) and 5 (*SCD5*) and the transcription factors sterol regulatory element binding transcription factor 1 (*SREBF1*), peroxisome proliferator-activated receptor gamma (*PPARG*), early growth response 2 (*EGR2*), and Sp1 transcription factor (*SP1*). To account for variations in RNA integrity and quantification and cDNA synthesis, mRNA abundance was normalized using the geometric mean of 3 reference genes: peptidylprolyl isomerase A (cyclophilin A; *PPIA*), ubiquitously expressed, prefoldin-like chaperone (*UXT*), and eukaryotic translation initiation factor 3, subunit K (*EIF3K*), which have been identified as suitable internal controls (Bionaz and Looor, 2007; Bonnet et al., 2013) and were confirmed as the most stable genes among those quantified using the geNorm procedure (Vandesompele et al., 2002). The primer sequences and qPCR performance are shown in Supplemental Tables S1 and S2, respectively (<http://dx.doi.org/10.3168/jds.2014-8731>). The abundance of gene transcripts was expressed as the $\log_2$ value of mRNA copy number relative to the geometric mean of the 3 reference genes.

Calculations and Statistical Analysis

Differences in the concentrations of *cis*-9 10:1, *cis*-9 12:1, and *cis*-9 14:1 (which are synthesized almost exclusively in the mammary gland through SCD activity; Bauman and Davis, 1974; Mosley and McGuire, 2007) measured on d 0 and the average over d 5 and 6 of Co administration were used as an indication of incomplete SCD inhibition. This allowed the endogenous synthesis of other SCD products in the mammary gland to be estimated (Griinari et al., 2000; Frutos et al., 2014).

All statistical analyses were performed using the SAS software package (version 9.3, SAS Institute Inc., Cary, NC). Animal performance and FA composition data were analyzed by one-way analysis of covariance using the MIXED procedure of SAS with a model that included the fixed effects of experimental treatment (mean values over d 5 and 6 of Co administration)

and measurements at the end of the adaptation period (mean values over d -2, -1, and 0 for animal performance and d 0 for FA composition) as a covariate. Data on milk FA composition recorded over the course of the experiment were subjected to ANOVA for repeated measures using the MIXED procedure of SAS and assuming a covariance structure fitted on the basis of Schwarz's Bayesian information model fit criterion. The statistical model included the fixed effects of treatment, experimental day, and their interaction, and the initial record measured on d 0 as a covariate. Measurements of gene mRNA abundances were analyzed by one-way ANOVA to test the fixed effect of treatment using the MIXED procedure of SAS. In all cases, animals were nested within the treatment and used as the error term to contrast the effect of Co administration. Relationships between the mRNA relative abundance (non-transformed data) of *SCD1* in mammary tissue and the percentage variations of mammary SCD indices were examined by regression analysis using the REG procedure of SAS. Differences were declared significant at $P < 0.05$ and a trend toward significance at $P > 0.05$ to 0.10. Covariate-adjusted least squares means are reported.

RESULTS

Animal Performance and Milk Composition

Neither DMI nor milk yield were significantly affected by the administration of Co-acetate ($P > 0.10$; Table 2). The same absence of differences occurred for milk fat, CP, lactose, and TS contents ($P > 0.10$).

Milk FA Composition

Milk concentrations of *cis*-9 10:1, *cis*-9 12:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 17:1, and *cis*-9 18:1 decreased ($P < 0.01$) in the Co9 treatment compared with the control (46, 55, 50, 47, 34, and 15%, respectively; Table 3). Similarly, the percentages of *trans* 18:2 and 18:3 isomers containing a *cis*-9 double bond, except for those of *cis*-9 *trans*-11 *cis*-15 18:3, were lowered following the administration of Co-acetate ($P < 0.05$). As shown in Figure 1, responses in milk FA were evident within 3 d of dosing and remained relatively stable during the treatment period.

Changes in milk FA containing a *cis*-9 double bond consistent with the inhibition of Δ^9 -desaturase were reflected in decreases ($P < 0.01$) of milk *cis*-9 10:1/10:0, *cis*-9 12:1/12:0, *cis*-9 14:1/14:0, *cis*-9 16:1/16:0, *cis*-9 17:1/17:0, *cis*-9 18:1/18:0, *cis*-9 *trans*-11 18:2/*trans*-11 18:1, *cis*-9 *trans*-12 18:2/*trans*-12 18:1, and *cis*-9 *trans*-11 *trans*-15 18:3/*trans*-11 *trans*-15 18:2 concen-

Table 2. Dry matter intake, and milk yield and composition in lactating ewes receiving 0 (control) or 9 (Co9) mg of Co/kg of BW per day

Item	Treatment ¹		SED ²	P-value
	Control	Co9		
DMI (g/d)	2,901	2,747	117.8	0.224
Yield (g/d)				
Milk	2,132	2,042	86.3	0.322
Fat	123.4	119.8	6.43	0.583
CP	110.4	104.7	4.14	0.195
Lactose	105.0	101.2	4.65	0.335
TS	356.9	342.5	13.11	0.300
Composition (g/kg)				
Fat	5.88	5.91	0.254	0.899
CP	5.21	5.19	0.079	0.752
Lactose	4.94	4.95	0.064	0.887
TS	16.86	16.85	0.277	0.979

¹Values represent the mean over d 5 and 6 of Co administration.²SED = standard error of the difference.

tration ratios by 45, 55, 52, 50, 33, 34, 42, 27, and 48%, respectively (Table 3). However, based on changes in milk FA that contain a *cis*-9 double bond and are almost exclusively synthesized de novo, this inhibition of SCD with Co-acetate is known to be incomplete, averaging 43, 51, and 49% for *cis*-9 10:1, *cis*-9 12:1, and *cis*-9 14:1, respectively. When these values were used as correction factors, it was estimated that approximately 28% of *cis*-9 18:1, 89% of *cis*-9 *trans*-11 18:2, 50% of *cis*-9 *trans*-12 18:2, 69% of *cis*-9 *trans*-13 18:2, and 100% of *cis*-9 *trans*-11 *trans*-15 18:3 in milk fat were synthesized endogenously.

Variations in the content of other milk FA included decreases in Δ^9 , 14 18:2, 20:4n-3, 20:4n-6, and 20:5n-3 ($P < 0.05$; Table 3), as well as in several odd-chain FA and minor medium-chain MUFA ($P < 0.05$; see Supplemental Table S3; <http://dx.doi.org/10.3168/jds.2014-8731>). However, Co had limited influence on the relative concentration of substrates for SCD, other than an increase in 18:0 ($P < 0.01$; Table 3). The sum of FA with <16, 16, and >16 carbons averaged 34.7 ± 0.47 , 23.8 ± 0.19 , and $30.5 \pm 1.59\%$ of total milk FA, respectively, and was not modified with the experimental treatment ($P > 0.10$; data not shown).

mRNA Abundance of Candidate Genes in Mammary Tissue

Oral administration of Co lowered the mRNA abundance of *SCD1* in the mammary secretory tissue ($P < 0.05$; Figure 2), with a reduction that averaged 33% (nontransformed data) and was correlated with the percentage variations of milk *cis*-9 10:1/10:0, *cis*-9 12:1/12:0, and *cis*-9 14:1/14:0 concentration ratios (Figure 3; $P < 0.01$). On the other hand, Co had no effect ($P > 0.10$) on the mRNA abundance of genes cod-

ing for *SCD5* and the transcription factors *SREBF1*, *PPARG*, *SP1*, and *EGR2*.

DISCUSSION

Previous investigations in lactating ruminants have shown the potential of Co administration to estimate mammary Δ^9 -desaturation through the changes in milk FA composition and the implied inhibition of SCD activity in cows (Shingfield et al., 2008; Taugbøl et al., 2008) and ewes (Frutos et al., 2014). Nevertheless, the mechanisms underlying the mode of action of Co remain uncertain.

Although the daily dose of Co used in this experiment (9 mg/kg of BW) would exceed the recommended maximum dietary levels set by the NRC (2005) for sheep, it was safely tolerated by the ewes, in agreement with a previous assay using the same TMR as in the present study but without lipid supplementation (Frutos et al., 2014). However, when comparing milk FA responses between both experiments, the reduction in SCD index by Co was greater and faster in the present study, with no significant differences between d 3, 5, and 6 of administration. Although the reason for this difference is not apparent, it may be related to the diet lipid composition. It is also unclear why lactating ewes are less responsive than cows, even when the latter receive the same or lower doses of Co per unit of BW (4.3–8.3 mg of Co/kg of BW; Taugbøl et al., 2010; Karlengen et al., 2012, 2013). Although this was supposed to be linked to variations between ruminant species in the absorption and transport of this element into the mammary epithelial cells, our results on mRNA abundance point to the existence of other interspecies differences in the effects of Co on SCD. Thus, in contrast to studies in cows suggesting that SCD inhibition is not

Table 3. Milk FA composition in lactating ewes receiving 0 (control) or 9 (Co9) mg of Co/kg of BW per day (effects on additional FA are reported in Supplemental Table S3, <http://dx.doi.org/10.3168/jds.2014-8731>)

FA (g/100 g of total FA)	Treatment ¹		SED ²	P-value
	Control	Co9		
SFA				
4:0	3.046	3.330	0.1722	0.134
6:0	2.645	2.736	0.1346	0.519
8:0	2.727	2.674	0.1791	0.774
10:0	8.204	7.780	0.4767	0.397
12:0	4.704	4.522	0.2109	0.411
14:0	11.424	12.029	0.3855	0.151
16:0	21.417	22.533	0.7818	0.187
17:0	0.515	0.520	0.0225	0.821
18:0	8.280	10.295	0.5503	0.005
20:0 ³	0.217	0.197	0.0100	0.085
22:0	0.104	0.089	0.0079	0.080
MUFA				
<i>cis</i> -9 10:1	0.304	0.163	0.0205	<0.001
<i>cis</i> -9 12:1	0.095	0.043	0.0080	<0.001
<i>cis</i> -9 14:1	0.242	0.122	0.0197	<0.001
<i>cis</i> -9 16:1	0.715	0.377	0.0526	<0.001
<i>cis</i> -9 17:1	0.178	0.117	0.0100	<0.001
<i>cis</i> -9 18:1	14.689	12.478	0.5503	0.003
<i>trans</i> -11 18:1	2.970	3.198	0.3868	0.569
<i>trans</i> -12 18:1	0.751	0.821	0.0512	0.204
18-carbon PUFA				
<i>cis</i> -9 <i>cis</i> -12 18:2	1.995	1.943	0.0665	0.456
<i>cis</i> -9 <i>trans</i> -11 18:2 ⁴	1.642	1.030	0.1914	0.011
<i>cis</i> -9 <i>trans</i> -12 18:2	0.171	0.135	0.0094	0.004
<i>cis</i> -9 <i>trans</i> -13 18:2	0.675	0.442	0.0382	<0.001
Δ 9,14 18:2	0.199	0.135	0.0141	0.002
<i>trans</i> -11 <i>cis</i> -15 18:2	0.477	0.469	0.0693	0.912
<i>trans</i> -11 <i>trans</i> -15 18:2	0.128	0.125	0.0176	0.890
<i>cis</i> -9 <i>cis</i> -12 <i>cis</i> -15 18:3	0.980	1.019	0.0538	0.492
<i>cis</i> -9 <i>trans</i> -11 <i>cis</i> -15 18:3	0.062	0.062	0.0076	0.998
<i>cis</i> -9 <i>trans</i> -11 <i>trans</i> -15 18:3	0.029	0.014	0.0022	<0.001
<i>cis</i> -9 <i>trans</i> -12 <i>cis</i> -15 18:3	0.016	0.010	0.0030	0.001
20- and 22-carbon PUFA				
20:3n-6	0.023	0.022	0.0016	0.490
20:3n-3	0.025	0.024	0.0041	0.853
20:4n-6	0.107	0.089	0.0039	0.001
20:4n-3 ⁵	0.067	0.052	0.0050	0.014
20:5n-3	0.054	0.042	0.0047	0.032
22:4n-6	0.024	0.020	0.0035	0.289
22:5n-6	0.029	0.022	0.0075	0.369
22:5n-3	0.100	0.092	0.0066	0.237
22:6n-3	0.040	0.038	0.0049	0.726
Δ^9 -desaturation ratio				
<i>cis</i> -9 10:1/10:0	0.038	0.021	0.0014	<0.001
<i>cis</i> -9 12:1/12:0	0.020	0.009	0.0009	<0.001
<i>cis</i> -9 14:1/14:0	0.021	0.010	0.0011	<0.001
<i>cis</i> -9 16:1/16:0	0.034	0.017	0.0016	<0.001
<i>cis</i> -9 17:1/17:0	0.342	0.230	0.0247	0.001
<i>cis</i> -9 18:1/18:0	1.869	1.233	0.0790	<0.001
<i>cis</i> -9 <i>trans</i> -11 18:2/ <i>trans</i> -11 18:1	0.513	0.297	0.0425	<0.001
<i>cis</i> -9 <i>trans</i> -12 18:2/ <i>trans</i> -12 18:1	0.230	0.169	0.0139	0.002
<i>cis</i> -9 <i>trans</i> -11 <i>trans</i> -15 18:3/ <i>trans</i> -11 <i>trans</i> -15 18:2	0.232	0.121	0.0421	0.030

¹Values represent the mean over d 5 and 6 of Co administration.²SED = standard error of the difference.³Coelutes with *cis*-9 *trans*-12 *trans*-15 18:3 and 18:3n-6.⁴Contains *trans*-8 *cis*-10 18:2 and *trans*-7 *cis*-9 18:2 as minor components.⁵Coelutes with 23:0.

Figure 1. Temporal changes in the concentration of stearoyl-CoA desaturase products in milk from lactating ewes receiving 0 (control) or 9 (Co9) mg of Co/kg of BW per day. Error bars represent the standard error of the mean (n = 6).

Figure 2. The mRNA relative abundance ($\log_2$ transformed data) of candidate genes in mammary tissue of lactating ewes receiving 0 (control) or 9 (Co9) mg of Co/kg of BW per day. Error bars represent the standard error of the mean ($n = 6$). *Differences ($P < 0.05$) between control and Co9 treatments.

mediated by changes in the abundance of its encoding genes (Karlengen et al., 2012; Vilkkil et al., 2012), we observed a significant decrease of 33% in *SCD1* expression that was related to the changes in milk FA concentration ratios that represent a proxy for SCD activity (Figure 3). The mean r value (0.76) for the relationship between both parameters was in the same range as those calculated from simultaneous measurements of mammary SCD indices and enzyme activities (Bernard et al., 2013). Assuming that these ratios are a suitable proxy of the desaturation of 10:0, 12:0, and 14:0 in the mammary gland, the relationship illustrated in Figure 3 represents a fairly strong evidence that the changes in the SCD product/substrate concentration ratios would be, at least partly, mediated by the decrease in *SCD1* mRNA abundance.

Between the 2 isoforms of SCD described in ruminants, *SCD1* is highly expressed in adipose tissues and mammary gland, but its regulation has not yet been well described (Bernard et al., 2013). Further, even less is known about *SCD5*, an isoform that is preferentially expressed in the brain and might also contribute to mammary desaturation (Bernard et al., 2013), although our results do not indicate any relevant role in the response to Co administration. The *SCD1* has been reported to be downregulated by *cis*-9 18:1, which is suggestive of a feedback mechanism (Kadegowda et al., 2009), whereas *trans*-11 18:1 or 16:0 increase its mRNA abundance (Kadegowda et al., 2009; Jin et al., 2012). Nevertheless, in our study, the milk concentration of *cis*-9 18:1 was reduced and that of *trans*-11 18:1 and 16:0 did not vary, which would indicate that changes in *SCD1* mRNA abundance were not due to changes in the enzyme products or substrates. Furthermore, the absence of differences in the analyzed transcription fac-

tors may support that they were not involved in the effect of Co on *SCD1*. However, these transcription regulators were chosen on the basis of their response to dietary factors and putative binding sites in the *SCD* gene promoter (Lengi and Corl, 2012; Bernard et al., 2013); therefore, the fact that they were not involved does not mean that the response of *SCD1* to Co dosing is not mediated by other transcription factors or posttranscriptional mechanisms. Thus far, Co has been hypothesized to inhibit SCD activity through a mechanism displacing Fe^{2+} from one of the enzyme complex components, either the cytochrome b_5 or the terminal desaturase, resulting in an iron-depleted SCD species (Shingfield et al., 2008; Taugbøl et al., 2010). Increased concentrations of Fe^{2+} in milk (Karlengen et al., 2013) might support this theory and possibly contribute to lower mammary *SCD1* mRNA abundance in ewes, probably by means of posttranscriptional mechanisms related to mRNA stability, as previously suggested in rodents (Rodriguez et al., 2007). In this regard, a better knowledge of the structural organization of the bovine and ovine *SCD1* transcription units (Ward et al., 1998; Chung et al., 2000), in particular the presence of iron responsive elements (**IRE**) in untranslated regions (Casey et al., 1988), would help to explain interspecies divergences in the effects of Co administration. In any event, differences between studies in biopsy collection and qPCR methods (Karlengen et al., 2012; Vilkkil et al., 2012) should neither be excluded. Further research would therefore be recommended to determine whether molecular mechanisms underlying the downregulation of *SCD1* in dairy ewes being drenched with Co are mediated by transcriptional or posttranscriptional processes.

In relation to the contribution of SCD to milk concentrations of FA containing a *cis*-9 double bond,

Figure 3. Relationship between the mRNA relative abundance (nontransformed data) of stearoyl-CoA desaturase 1 (*SCD1*) in mammary tissue and the percentage variations of milk *cis*-9 10:1/10:0, *cis*-9 12:1/12:0, and *cis*-9 14:1/14:0 concentration ratios in lactating ewes receiving 0 (control) or 9 (Co9) mg of Co/kg of BW per day.

available studies on the endogenous synthesis of *cis*-9 *trans*-11 18:2 in dairy ewes have shown highly variable results. As mentioned above, mean estimates of 51 and 74% of milk *cis*-9 *trans*-11 18:2 deriving from SCD activity were obtained with Co-acetate and sterculic acid administration, respectively (Bichi et al., 2012; Frutos et al., 2014). Despite differences in the methodological approaches could not be excluded, the observed variation appeared to be attributable to diet composition (nonsupplemented TMR vs. pasture; i.e., supplying mainly 18:2n-6 or 18:3n-3, respectively) and their potential effect on rumen lipid metabolism (AbuGhazaleh et al., 2003; Toral et al., 2012b). In this regard, the high proportion of endogenous *cis*-9 *trans*-11 18:2 that we found in this study (89%) is consistent with a greater supply of *trans*-11 18:1 of ruminal origin due to the presence in the diet of an 18:3n-3-rich oil (AbuGhazaleh et al., 2003; Loor et al., 2005). In addition, PUFA from linseed oil escaping ruminal metabolism or their biohydrogenation intermediates may also inhibit *SCD* gene expression or activity (Bernard et al., 2013), thereby contributing to the variation between studies. Altogether, both mechanisms would support that basal diet composition is a major determinant of the relative contribution of Δ^9 -desaturation to milk rumenic acid content. Thus, given that the percentage of *trans*-11 18:1 converted to *cis*-9 *trans*-11 18:2 appears to be independent of postruminal supply (Shingfield et al., 2007), the higher the supply of *trans*-11 18:1, the higher the proportion of *cis*-9 *trans*-11 18:2 in milk from endogenous synthesis.

However, the estimated percentage of 18:0 desaturated to *cis*-9 18:1 (on average, 35%) was lower than previously reported in ewes (48–57%; Bichi et al., 2012; Frutos et al., 2014), cows (on average, 54%; Glasser et al., 2008), and goats (63–70%; Annison et al., 1967), and the same occurred with the proportion of milk *cis*-9 18:1 synthesized endogenously (29%) in comparison with most in vivo estimates in these 3 species (45–84%; e.g., Annison et al., 1967; Mosley and McGuire, 2007; Bichi et al., 2012). The reasons for these inconsistencies between studies are not clear but may also lie in different diet compositions, as suggested by the very low endogenous synthesis of *cis*-9 18:1 observed after its abomasal infusion in cows (11–16%; Enjalbert et al., 1998; Chelikani et al., 2004). Thus, it may be speculated that part of the *cis*-9 18:1 supplied with the linseed oil (18% of total FA) would have escaped ruminal biohydrogenation, increasing its duodenal flow and resulting in higher *cis*-9 18:1 uptake by the mammary tissue, compared with previous assays in sheep fed diets with a lower oleic acid content (Bichi et al., 2012; Frutos et al., 2014). Although the transfer of absorbed *cis*-9 *trans*-11 18:2 into milk has been

demonstrated to be more efficient than desaturation from *trans*-11 18:1 (Shingfield et al., 2007), few studies have been published for *cis*-9 18:1. Nevertheless, the available information seems to indicate similar results (Enjalbert et al., 1998). Another explanation for the low proportion of oleic acid synthesized endogenously may be related to the main role of 18:0 desaturation in the reduction of the milk fat melting point to maintain an adequate fluidity and subsequent secretion (Bernard et al., 2013). In this regard, it may be speculated that changes in the milk FA profile caused by dietary linseed oil (i.e., increases in PUFA and decreases in medium-chain SFA concentrations) would have already lowered the melting point and therefore minimized the relative importance of the conversion to *cis*-9 18:1 to allow milk fat secretion.

Diet supplementation with linseed oil promoted the secretion in milk of some minor *cis*-9-containing PUFA associated with 18:3n-3 metabolism (Loor et al., 2005; Lerch et al., 2012), which consequently favored their quantification and subsequent discrimination as potential SCD products. Thus, as shown in Figure 1, temporal changes in the concentration of *cis*-9 *trans*-11 *trans*-15 18:3 in the control versus Co9 animals confirms that it is derived exclusively from Δ^9 -desaturation, whereas the lack of variation in *cis*-9 *trans*-11 *cis*-15 18:3 supports a ruminal origin (Bichi et al., 2012; Lerch et al., 2012). To our knowledge, *cis*-9 *trans*-12 *cis*-15 18:3 has not been described to date as a product of SCD activity. However, differences in its temporal changes suggest that it would mainly come from Δ^9 -desaturation (60%).

Finally, the milk proportion of Δ^9 ,14 18:2 was significantly decreased by Co-acetate administration, in agreement with an endogenous synthesis (76%) by SCD action. Therefore, although its double bond geometry cannot be assigned with the chromatographic procedures used, it may be hypothesized that this isomer corresponds to *cis*-9 *trans*-14 18:2, a minor FA that has been related to alternative pathways of ruminal biohydrogenation (Vazirigohar et al., 2014).

CONCLUSIONS

Short-term administration of Co-acetate to lactating sheep reduces mammary SCD indices and may represent a cost-effective method to study the relevance of this enzyme in milk FA composition. Although the mode of action of Co is still uncertain, the lowered mRNA abundance of *SCD1* detected in the mammary secretory tissue of ewes drenched with Co suggests that it would be at least partly mediated by the downregulation of this candidate gene. The observed high proportion of endogenous *cis*-9 *trans*-11 18:2 (89%) and low of *cis*-9 18:1 (29%) may be explained by the presence of dietary

linseed oil, which would support basal diet composition as a major determinant of the relative contribution of Δ^9 -desaturation to the endogenous synthesis of FA in the mammary gland.

ACKNOWLEDGMENTS

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO; AGL2011-23700). P. G. Toral gratefully acknowledges receipt of a postdoctoral research contract from the MINECO (Juan de la Cierva program).

REFERENCES

- AbuGhazaleh, A. A., D. J. Schingoethe, A. R. Hippen, and K. F. Kalscheur. 2003. Conjugated linoleic acid and vaccenic acid in rumen, plasma, and milk of cows fed fish oil and fats differing in saturation of 18 carbon fatty acids. *J. Dairy Sci.* 86:3648–3660.
- Annisson, E. F., J. L. Linzell, S. Fazakerley, and B. W. Nichols. 1967. The oxidation and utilization of palmitate, stearate, oleate and acetate by the mammary gland of the fed goat in relation to their overall metabolism, and the role of plasma phospholipids and neutral lipids in milk-fat synthesis. *Biochem. J.* 102:637–647.
- Bauman, D. E., and C. L. Davis. 1974. Biosynthesis of milk fat. Pages 31–75 in *Lactation: A Comprehensive Treatise*. Vol. 2. B. L. Larson and V. R. Smith, ed. Academic Press, New York, NY.
- Bernard, L., C. Leroux, and Y. Chilliard. 2013. Expression and nutritional regulation of stearoyl-CoA desaturase genes in the ruminant mammary gland: Relationship with milk fatty acid composition. Pages 161–193 in *Stearoyl-CoA Desaturase Genes in Lipid Metabolism*. J. M. Ntambi, ed. Springer Science+Business Media, New York, NY.
- Bichi, E., G. Hervás, P. G. Toral, J. J. Loor, and P. Frutos. 2013. Milk fat depression induced by dietary marine algae in dairy ewes: Persistency of milk fatty acid composition and animal performance responses. *J. Dairy Sci.* 96:524–532.
- Bichi, E., P. G. Toral, G. Hervás, P. Frutos, P. Gómez-Cortés, M. Juárez, and M. A. de la Fuente. 2012. Inhibition of Δ^9 -desaturase activity with sterculic acid: Effect on the endogenous synthesis of *cis*-9 18:1 and *cis*-9 *trans*-11 18:2 in dairy sheep. *J. Dairy Sci.* 95:5242–5252.
- Bionaz, M., and J. J. Loor. 2007. Identification of reference genes for quantitative real-time PCR in the bovine mammary gland during the lactation cycle. *Physiol. Genomics* 29:312–319.
- Bonnet, M., L. Bernard, S. Bes, and C. Leroux. 2013. Selection of reference genes for quantitative real-time PCR normalisation in adipose tissue, muscle, liver and mammary gland from ruminants. *Animal* 7:1344–1353.
- Casey, J. L., M. W. Hentze, D. M. Koeller, S. W. Caughman, T. A. Rouault, R. D. Klausner, and J. B. Harford. 1988. Iron-responsive elements: Regulatory RNA sequences that control mRNA levels and translation. *Science* 240:924–928.
- Chelikani, P. K., J. A. Bell, and J. J. Kennelly. 2004. Effects of feeding or abomasal infusion of canola oil in Holstein cows 1. Nutrient digestion and milk composition. *J. Dairy Res.* 71:279–287.
- Chung, M., S. Ha, S. Jeong, J. Bok, K. Cho, M. Baik, and Y. Choi. 2000. Cloning and characterization of bovine stearoyl CoA desaturase cDNA from adipose tissues. *Biosci. Biotechnol. Biochem.* 64:1526–1530.
- Enjalbert, F., M. C. Nicot, C. Bayourthe, and R. Moncoulon. 1998. Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. *J. Nutr.* 128:1525–1532.
- Frutos, P., P. G. Toral, E. Ramos-Morales, K. J. Shingfield, A. Belenguier, and G. Hervás. 2014. Oral administration of cobalt acetate alters milk fatty acid composition, consistent with an inhibition of

- stearoyl-coenzyme A desaturase in lactating ewes. *J. Dairy Sci.* 97:1036–1046.
- Glasser, F., A. Ferlay, M. Doreau, P. Schmidely, D. Sauvant, and Y. Chilliard. 2008. Long-chain fatty acid metabolism in dairy cows: A meta-analysis of milk fatty acid yield in relation to duodenal flows and de novo synthesis. *J. Dairy Sci.* 91:2771–2785.
- Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9 -desaturase. *J. Nutr.* 130:2285–2291.
- Halmemies-Beauchet-Filleau, A., T. Kokkonen, A. M. Lampi, V. Toivonen, K. J. Shingfield, and A. Vanhatalo. 2011. Effect of plant oils and camelina expeller on milk fatty acid composition in lactating cows fed diets based on red clover silage. *J. Dairy Sci.* 94:4413–4430.
- Jin, Y. C., Z. H. Li, Z. S. Hong, C. X. Xu, J. A. Han, S. H. Choi, J. L. Yin, Q. K. Zhang, K. B. Lee, S. K. Kang, M. K. Song, Y. J. Kim, H. S. Kang, Y. J. Choi, and H. G. Lee. 2012. Conjugated linoleic acid synthesis-related protein proteasome subunit α 5 (PSMA5) is increased by vaccenic acid treatment in goat mammary tissue. *J. Dairy Sci.* 95:4286–4297.
- Kadegowda, A. K. G., M. Bionaz, L. S. Piperova, R. A. Erdman, and J. J. Looor. 2009. Peroxisome proliferator-activated receptor- γ activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents. *J. Dairy Sci.* 92:4276–4289.
- Karlengen, I. J., O. M. Harstad, O. Taubøl, I. Berget, A. H. Aastveit, and D. I. Våge. 2012. The effect of excess cobalt on milk fatty acid profiles and transcriptional regulation of *SCD*, *FASN*, *DGAT1* and *DGAT2* in the mammary gland of lactating dairy cows. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 96:1065–1073.
- Karlengen, I. J., O. Taubøl, B. Salbu, A. H. Aastveit, and O. M. Harstad. 2013. Effect of different levels of supplied cobalt on the fatty acid composition of bovine milk. *Br. J. Nutr.* 109:834–843.
- Lengi, A. J., and B. A. Corl. 2012. Regulation of the bovine SCD5 promoter by EGR2 and SREBP1. *Biochem. Biophys. Res. Commun.* 421:375–379.
- Lerch, S., K. J. Shingfield, A. Ferlay, A. Vanhatalo, and Y. Chilliard. 2012. Rapeseed or linseed in grass-based diets: Effects on conjugated linoleic and conjugated linolenic acid isomers in milk fat from Holstein cows over 2 consecutive lactations. *J. Dairy Sci.* 95:7269–7287.
- Lock, A. L., and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids* 39:1197–1206.
- Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2005. Intestinal flow and digestibility of *trans* fatty acids and conjugated linoleic acids (CLA) in dairy cows fed a high-concentrate diet supplemented with fish oil, linseed oil, or sunflower oil. *Anim. Feed Sci. Technol.* 119:203–225.
- Mosley, E. E., and M. A. McGuire. 2007. Methodology for the in vivo measurement of the Δ^9 -desaturation of myristic, palmitic, and stearic acids in lactating dairy cattle. *Lipids* 42:939–945.
- NRC. 2005. Mineral Tolerance of Animals. 2nd rev. ed. Natl. Acad. Press, Washington, DC.
- Rodriguez, A., M. Hilvo, L. Kytomaki, R. Fleming, R. Britton, B. Bacon, and S. Parkkila. 2007. Effects of iron loading on muscle: Genome-wide mRNA expression profiling in the mouse. *BMC Genomics* 8:379.
- Shingfield, K. J., S. Ahvenjärvi, V. Toivonen, A. Äröla, K. V. V. Nurmela, P. Huhtanen, and J. M. Griinari. 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Anim. Sci.* 77:165–179.
- Shingfield, K. J., S. Ahvenjärvi, V. Toivonen, A. Vanhatalo, and P. Huhtanen. 2007. Transfer of absorbed *cis*-9, *trans*-11 conjugated linoleic acid into milk is biologically more efficient than endogenous synthesis from absorbed vaccenic acid in lactating cows. *J. Nutr.* 137:1154–1160.
- Shingfield, K. J., A. Äröla, S. Ahvenjärvi, A. Vanhatalo, V. Toivonen, J. M. Griinari, and P. Huhtanen. 2008. Ruminal infusions of cobalt-EDTA reduce mammary Δ^9 -desaturase index and alter milk fatty acid composition in lactating cows. *J. Nutr.* 138:710–717.
- Shingfield, K. J., M. Bonnet, and N. D. Scollan. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. *Animal* 7(Suppl. 1):132–162.
- Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty-acid content and composition of feedstuffs and feces. *J. Agric. Food Chem.* 36:1202–1206.
- Taubøl, O., I. J. Karlengen, T. Bolstad, A. H. Aastveit, and O. M. Harstad. 2008. Cobalt supplied per os reduces the mammary Δ^9 -desaturase index of bovine milk. *J. Anim. Sci.* 86:3062–3068.
- Taubøl, O., I. J. Karlengen, B. Salbu, A. H. Aastveit, and O. M. Harstad. 2010. Intravenous injections of cobalt reduce fatty acid desaturation products in milk and blood of lactating cows. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 94:635–640.
- Toral, P. G., A. Belenguer, K. J. Shingfield, G. Hervás, V. Toivonen, and P. Frutos. 2012b. Fatty acid composition and bacterial community changes in the rumen fluid of lactating sheep fed sunflower oil plus incremental levels of marine algae. *J. Dairy Sci.* 95:794–806.
- Toral, P. G., Y. Chilliard, and L. Bernard. 2012a. Short communication: In vivo deposition of [$1\text{-}^{13}\text{C}$]vaccenic acid and the product of its Δ^9 -desaturation, [$1\text{-}^{13}\text{C}$]rumenic acid, in the body tissues of lactating goats fed oils. *J. Dairy Sci.* 95:6755–6759.
- Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology* 3:RESEARCH0034.
- Vazirigohar, M., M. Dehghan-Banadaky, K. Rezayazdi, S. J. Krizsan, A. Nejati-Javaremi, and K. J. Shingfield. 2014. Fat source and dietary forage-to-concentrate ratio influences milk fatty-acid composition in lactating cows. *Animal* 8:163–174.
- Vilkki, J., S. Viitala, H. Leskinen, J. Taponen, L. Bernard, and K. J. Shingfield. 2012. Effect of ruminal cobalt-EDTA infusion on milk fat composition and mammary lipogenic gene expression. Page 238 in Book of Abstracts of the 63rd Annual Meeting of the European Federation of Animal Science. Bratislava, Slovakia.
- Ward, R. J., M. T. Travers, S. E. Richards, R. G. Vernon, A. M. Salter, P. J. Buttery, and M. C. Barber. 1998. Stearoyl-CoA desaturase mRNA is transcribed from a single gene in the ovine genome. *Biochim. Biophys. Acta* 1391:145–156.