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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

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ABSTRACT

This study was conducted in lactating ewes to examine the involvement of Δ^9-desaturase in mammary lipogenesis, especially in the endogenous synthesis of *cis*-9, *trans*-11 18:2 and *cis*-9 18:1, because no information on this matter was available for dairy sheep. With this aim, 6 Assaf ewes were monitored in a 15-d experiment, which included a 5-d pretreatment period, a 5-d treatment period, and a 5-d posttreatment period. During the treatment period, ewes received 0.5 g/d of sterculic acid (a cyclopropene fatty acid that inhibits Δ^9-desaturase), delivered intravenously in 4 equal doses at 6-h intervals. Animals were fed pasture to supply mainly α-linolenic acid and minimize the amount of milk *cis*-9, *trans*-11 18:2 of ruminal origin. Sterculic acid administration was calculated to inhibit Δ^9-desaturase by 70% based on the milk content of *cis*-9 14:1. This inhibition resulted in decreases in the milk content of the enzyme products (e.g., *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1, and *cis*-9, *trans*-11 18:2) and increases in its substrates (e.g., 14:0, 18:0, and *trans*-11 18:1), as well as in reductions in the desaturase indexes. Some other milk fatty acids, further to previously reported products or substrates of Δ^9-desaturase (e.g., *cis*-15 18:1 and *cis*-9, *cis*-15 18:2, or *trans*-11, *trans*-15 18:2, and *cis*-9, *trans*-11, *trans*-15 18:3), were also affected by sterculic acid administration. Endogenous synthesis was the major source of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2, accounting for 63 and 74% of its content in milk fat, respectively. To our knowledge, the present study provides the first estimates of endogenous synthesis of these 2 bioactive fatty acids in ovine milk fat.

Key words: desaturase system, cyclopropene fatty acid, oleic acid, rumenic acid

INTRODUCTION

In ruminants, the enzyme Δ^9-desaturase has a key role in the synthesis of some milk FA through the introduction of a *cis* double bond between carbons 9 and 10 (Palmquist et al., 2005). Since Griinari et al. (2000) demonstrated that endogenous synthesis of the potentially health-promoting *cis*-9, *trans*-11 conjugated linoleic acid (**CLA**) from *trans*-11 18:1 of ruminal origin represents the primary source of CLA in the milk fat of lactating cows, the study of Δ^9-desaturase has had a renewed interest.

However, available information on the role of Δ^9-desaturase in mammary lipogenesis is still scarce in cows and goats and is almost nonexistent in sheep, despite the fact that a putatively higher activity of this enzyme has been suggested in the latter species based on differences in its mRNA abundance (Tsiplakou et al., 2009).

In addition, the number of studies quantifying the activity of Δ^9-desaturase on other substrates besides *cis*-9, *trans*-11 CLA is very limited. This is especially notable in relation to the synthesis of *cis*-9 18:1 (oleic acid) from 18:0 desaturation (Bickerstaffe and Johnson, 1972; Jeffcoat and Pollard, 1977; Mosley and McGuire, 2007), in spite of the relevance of its role in decreasing the milk fat melting point and probably preventing milk fat depression (Shingfield and Griinari, 2007).

Available studies estimating the activity of Δ^9-desaturase in ruminants include the use of direct methods, by means of a tracer (for example, [1-^{13}C]*trans*-11 18:1; Mosley et al., 2006; Bernard et al., 2010), and indirect methods, through quantification of duodenal and milk FA flows (Shingfield et al., 2007; Glasser et al., 2008) or inhibition of the desaturase system (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004). The present study was conducted in lactating ewes with the aim of examining the involvement of Δ^9-desaturase in mammary lipogenesis, using a cyclopropene FA (sterculic acid) that inhibits the enzymatic system (Gomez et al.,

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2003). Special attention was paid to the endogenous synthesis of *cis*-9, *trans*-11 18:2 and *cis*-9 18:1 in the mammary gland.

MATERIALS AND METHODS

Animals, Experimental Design, and Management

All experimental procedures were performed in accordance with the Spanish Royal Decree 1201/2005 for the protection of animals used for experimental and other scientific purposes.

Six primiparous Assaf ewes (73.3 ± 3.30 kg of BW) in midlactation (94 ± 1.5 DIM at the beginning of the experiment) were used. Sheep were housed in individual tie stalls and fed ad libitum a pasture diet to supply mainly 18:3n-3 (α-linolenic acid) and minimize the amount of milk *cis*-9, *trans*-11 18:2 of ruminal origin. The pasture was harvested from an irrigated sward of *Lolium perenne*, *Trifolium pratense*, and *Dactylis glomerata* before commencing the trial and kept frozen at −30°C until the evening before being used. After thawing at approximately 4 to 10°C, the pasture was offered twice daily at 0900 and 1900 h.

Before the experiment, all animals were allowed to graze pasture (a plot of the same sward described above) for 4 wk, followed by another week of adaptation to indoor conditions. The 15-d experiment consisted of a pretreatment period (d 1 to 5), a treatment period (d 6 to 10), and a posttreatment period (d 11 to 15). During the 5-d treatment period, the ewes received 0.5 g/d of chemically synthesized sterculic acid (Planta Piloto de Química Fina, University of Alcalá, Alcalá de Henares, Spain), suspended in 6 mL of 10% Intralipid (Fresenius Kabi SA, Barcelona, Spain) and 0.5 g of Simulsol 5817 (Seppic, Paris, France) and brought to 7 mL with 0.9% (wt/vol) saline solution (B. Braun Medical SA, Barcelona, Spain). The mixture was sonicated at 100 A for 1 min to ensure thorough mixing. One-fourth of the daily treatment dose was delivered every 6 h by jugular infusion.

Ewes were milked twice daily at approximately 0830 and 1830 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain) and had continuous access to clean water and a vitamin-mineral supplement (Tegablock, Inatega, León, Spain).

Measurements, Sampling Procedures, and Chemical Analyses

During the experimental period, DMI was recorded daily by weighing the amount of pasture DM offered and refused by each ewe. Samples of the pasture were collected at harvest, freeze-dried, and stored at −30°C until they were analyzed for DM (ISO 6496:1999; ISO, 1999a), ash (ISO 5984:2002; ISO, 2002a), and CP (ISO 5983-2:2009; ISO, 2009). Neutral and acid detergent fibers were determined as described by Mertens (2002) and AOAC (2006; official method 973.18), respectively, using an Ankom^{2000} Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Neutral detergent fiber was assayed with sodium sulfite and α-amylase and expressed with residual ash (the latter also for ADF). The content of ether extract in the diets was determined by Ankom Filter Bag Technology (procedure Am 5-04; AOCS, 2008).

The fat in the pasture was extracted following AOAC (2006) official methods (969.33 and 963.22) and then methylated and analyzed by gas chromatography, under the same conditions as described below for milk FA methyl esters (**FAME**).

Individual milk yield was recorded daily, and milk samples were collected from each animal and composited according to morning and evening milk yield. One aliquot of milk was treated with natamycin and stored at 4°C until analyzed for fat, protein, lactose, and TS content by infrared spectrophotometry (ISO 9622:1999; ISO, 1999b), using a MilkoScan 255 A/S N (Foss Electric, Hillerød, Denmark). Milk FA composition was determined in untreated aliquots that were stored at −30°C until analysis. Milk fat was extracted as described by Luna et al. (2005), and FAME were prepared by base-catalyzed methanolysis of the glycerides (ISO 15884:2002; ISO, 2002b). Analysis of FAME in hexane was performed on a gas chromatograph (Agilent 6890 N Network System; Agilent, Palo Alto, CA) with auto injector and fitted with a flame-ionization detector. The FAME profile was determined by split injection (1:100) onto a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d., 0.20-μm film thickness; Varian, Middelburg, the Netherlands) using a gradient temperature program. The initial oven temperature was 160°C. After 80 min, it was increased at 10°C/min to 210°C and then held for 35 min. Helium was the carrier gas, and the injector and detector temperatures were 250°C. Quantification of individual FAME was made by reference to a milk fat with a known composition (CRM 164; European Community Bureau of Reference, Brussels, Belgium). Individual CLA isomers were identified by comparison with standard mixtures distributed by Nu-Chek Prep. Inc. (Elysian, MN). Standard GLC-461 from Nu-Chek Prep Inc. was also used to identify other FA.

Calculations and Statistical Analysis

The content of *cis*-9 14:1 in the pretreatment period and on the last day of sterculic acid administration

Table 1. Chemical composition and FA profile of the pasture (n = 9)

Item	Pasture	SEM
Chemical composition, g/kg of DM		
DM[1]	255	0.7
OM	932	0.2
CP	136	0.4
NDF	460	0.7
ADF	252	0.4
Ether extract	33	0.1
FA profile, % of total FA methyl esters		
16:0	12.4	0.16
18:0	1.5	0.09
cis-9 18:1	4.3	0.26
cis-9, *cis*-12 18:2	14.1	0.51
cis-9, *cis*-12, *cis*-15 18:3	55.4	0.82
20:0	1.5	0.11
22:0	3.1	0.12
22:2	4.5	0.28

[1]Dry matter is in grams per kilogram of fresh matter.

was used to calculate a correction factor for incomplete inhibition of Δ^9-desaturase, to estimate the endogenous synthesis of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2 (Griinari et al., 2000). Based on previous studies in lactating cows (Mosley and McGuire, 2007), which estimated that approximately 90% of *cis*-9 14:1 was synthesized in the mammary gland via Δ^9-desaturation, it was assumed that the mammary gland was also the major site for conversion of 14:0 to *cis*-9 14:1 in lactating sheep. Desaturase indexes were calculated as follows: product of Δ^9-desaturase/(product of Δ^9-desaturase + substrate of Δ^9-desaturase).

Data were evaluated by repeated measurement analysis using the MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC) and assuming a covariance structure on the basis of Schwarz's Bayesian information model fit criterion. The statistical model included the fixed effect of period, animal was considered a random effect, and day, nested in period, appeared in the repeated statement.

Least squares means are reported throughout. Significance differences were declared at $P < 0.05$ and tendencies accepted if $P < 0.10$.

RESULTS

Pasture Composition

Chemical composition of the pasture, including its FA profile, is presented in Table 1. α-Linolenic acid was the most abundant FA present in pasture (55.4%), followed by linoleic (14.1%) and palmitic (12.4%) acids. None of the other FA represented more than 5% of total FAME.

Animal Performance and Milk Composition

As shown in Table 2, sterculic acid did not affect DMI during its administration but caused a 7% decrease in the posttreatment period ($P = 0.004$). No changes were found in milk yield. However, a progressive decrease ($P < 0.001$) in milk fat production was observed throughout the trial, in association with reductions in milk fat content during the treatment and posttreatment periods (−5 and −13%, respectively, relative to the mean value of the pretreatment period). Infusion of sterculic acid had only a slight effect on milk protein content (−2.3%; $P < 0.001$), both during and after administration, whereas lactose percentage was minimally increased in the posttreatment period (+1.3%; $P < 0.001$).

Table 2. Dry matter intake, milk yield, and milk composition in dairy ewes before (pretreatment), during (treatment), and after (posttreatment) sterculic acid administration

Item	Pretreatment	Treatment	Posttreatment	SED[1]	*P*-value[2]
DMI, g/d	2,345[a]	2,360[a]	2,188[b]	55.9	0.004
Yield, g/d					
Milk	1,137	1,136	1,135	22.5	0.994
Fat	68.7[a]	64.4[b]	61.2[c]	1.54	0.001
Protein	59.3	58.0	57.9	1.23	0.447
Lactose	55.7	55.6	56.2	1.08	0.855
TS	193.3	189.1	187.4	4.52	0.435
Composition, g/100 g of raw milk					
Fat	6.03[a]	5.71[b]	5.26[c]	0.122	<0.001
Protein	5.23[a]	5.12[b]	5.10[b]	0.029	<0.001
Lactose	4.89[b]	4.88[b]	4.95[a]	0.018	<0.001
TS	16.96[a]	16.62[b]	16.12[c]	0.115	<0.001

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

[1]SED = SE of the difference.

[2]Probability of significant differences between periods.

Milk FA Composition

Treatment with sterculic acid reduced ($P < 0.001$) the indices of Δ^9-desaturase activity shown in Table 3. The greatest decrease (−71%, always in comparison with the pretreatment period) corresponded to the index of *cis*-9 14:1 and 14:0, although the changes observed for *cis*-9 10:1 and 10:0, *cis*-9 16:1 and 16:0, *cis*-9 18:1 and 18:0, *cis*-9, *trans*-11 18:2 and *trans*-11 18:1, and *cis*-9, *trans*-11, *trans*-15 18:3 and *trans*-11, *trans*-15 18:2 were also substantial (−47, −28, −37, −42, and −46%, respectively). The initial values were not recovered during the posttreatment period ($P < 0.001$).

Similarly, the reductions ($P < 0.001$) in concentrations of the Δ^9-desaturase products persisted partially into the posttreatment period (see Figure 1). According to the temporal variations, the inhibition of Δ^9-desaturase activity reached its maximum value around the second or third day on treatment (d 7 or 8 of the experiment), but its recovery was slower and the values in the pretreatment period were achieved only for *cis*-9 18:1 and on the last day of the experiment (d 15; Figure 1). Comparable changes (i.e., significant reductions that persisted partially after the administration) were also observed for *cis*-9 10:1, *cis*-9 17:1, *cis*-9, *cis*-15 18:2, *cis*-9, *trans*-12 18:2, and *cis*-9, *trans*-13 18:2 (Figure 2 and Table 4).

The inhibition of Δ^9-desaturase induced an increase ($P \leq 0.001$) in the relative concentration of 14:0 (+6%), 18:0 (+57%), *trans*-11 18:1 (+16%), and *trans*-11, *trans*-15 18:2 (+20%), with temporal patterns of variation that were consistent with the persistency of the response (Figure 1). However, the percentages of 10:0 and 16:0 in milk were not affected (Table 4).

The activity of Δ^9-desaturase was calculated to be reduced by 70% with sterculic acid administration. When the data from the last day of sterculic acid administration were used, the endogenous synthesis of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2 was estimated to account for 63 and 74%, respectively, of their content in milk fat.

Regarding other FA, some increased during the treatment period (e.g., *cis*-15 18:1, *trans*-12 18:1, *cis*-9, *cis*-12 18:2, *cis*-9, *cis*-12, *cis*-15 18:3), whereas others decreased (e.g., *cis*-5, *cis*-8, *cis*-11, *cis*-14 20:4) or showed a tendency to decrease (e.g., *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5). As expected, the proportion of SFA was increased with the administration of sterculic acid, whereas those of MUFA and PUFA were decreased. Linoleic and α-linolenic acids achieved the highest concentration in the posttreatment period (+20 and +32%, respectively), whereas the proportion of FA synthesized de novo or preformed was not affected by treatment ($P > 0.10$; Table 3).

Table 3. Indices of desaturase activity and major classes of FA of milk in dairy ewes before (pretreatment), during (treatment), and after (posttreatment) sterculic acid administration

Item	Pretreatment	Treatment	Posttreatment	SED[1]	P-value[2]
Desaturase index					
cis-9 10:1/(*cis*-9 10:1 + 10:0)	0.040[a]	0.022[c]	0.026[b]	0.0007	<0.001
cis-9 14:1/(*cis*-9 14:1 + 14:0)	0.014[a]	0.005[c]	0.006[b]	0.0005	<0.001
cis-9 16:1/(*cis*-9 16:1 + 16:0)	0.053[a]	0.039[b]	0.041[b]	0.0014	<0.001
cis-9 18:1/(*cis*-9 18:1 + 18:0)	0.636[a]	0.405[c]	0.561[b]	0.0082	<0.001
cis-9, *trans*-11 18:2/(*cis*-9, *trans*-11 18:2 + *trans*-11 18:1)	0.316[a]	0.182[c]	0.221[b]	0.0087	<0.001
cis-9, *trans*-11, *trans*-15 18:3/(*cis*-9, *trans*-11, *trans*-15 18:3 + *trans*-11, *trans*-15 18:2)	0.351[a]	0.193[c]	0.240[b]	0.0083	<0.001
cis-9, *trans*-11, *cis*-15 18:3/(*cis*-9, *trans*-11, *cis*-15 18:3 + *trans*-11, *cis*-15 18:2)	0.400	0.377	0.358	0.0240	0.216
According to degree of saturation, g/100 g of total FA methyl esters					
SFA	66.87[c]	74.32[a]	70.87[b]	0.692	<0.001
MUFA	24.93[a]	18.00[c]	20.82[b]	0.494	<0.001
PUFA	6.13[b]	5.91[b]	6.42[a]	0.143	0.005
According to origin,[3] g/100 g of total FA methyl esters					
Σ 6–14-carbon FA	29.42	29.80	29.49	0.520	0.745
Σ 16-carbon FA	24.64	24.49	24.43	0.214	0.580
Σ ≥18-carbon FA	37.99	37.46	38.11	0.627	0.544

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

[1]SED = SE of the difference.

[2]Probability of significant differences between periods.

[3]The 6–14-carbon FA represent de novo-synthesized FA, ≥18-carbon FA represent preformed FA taken up from circulation, and 16-carbon FA represent FA derived from both sources.

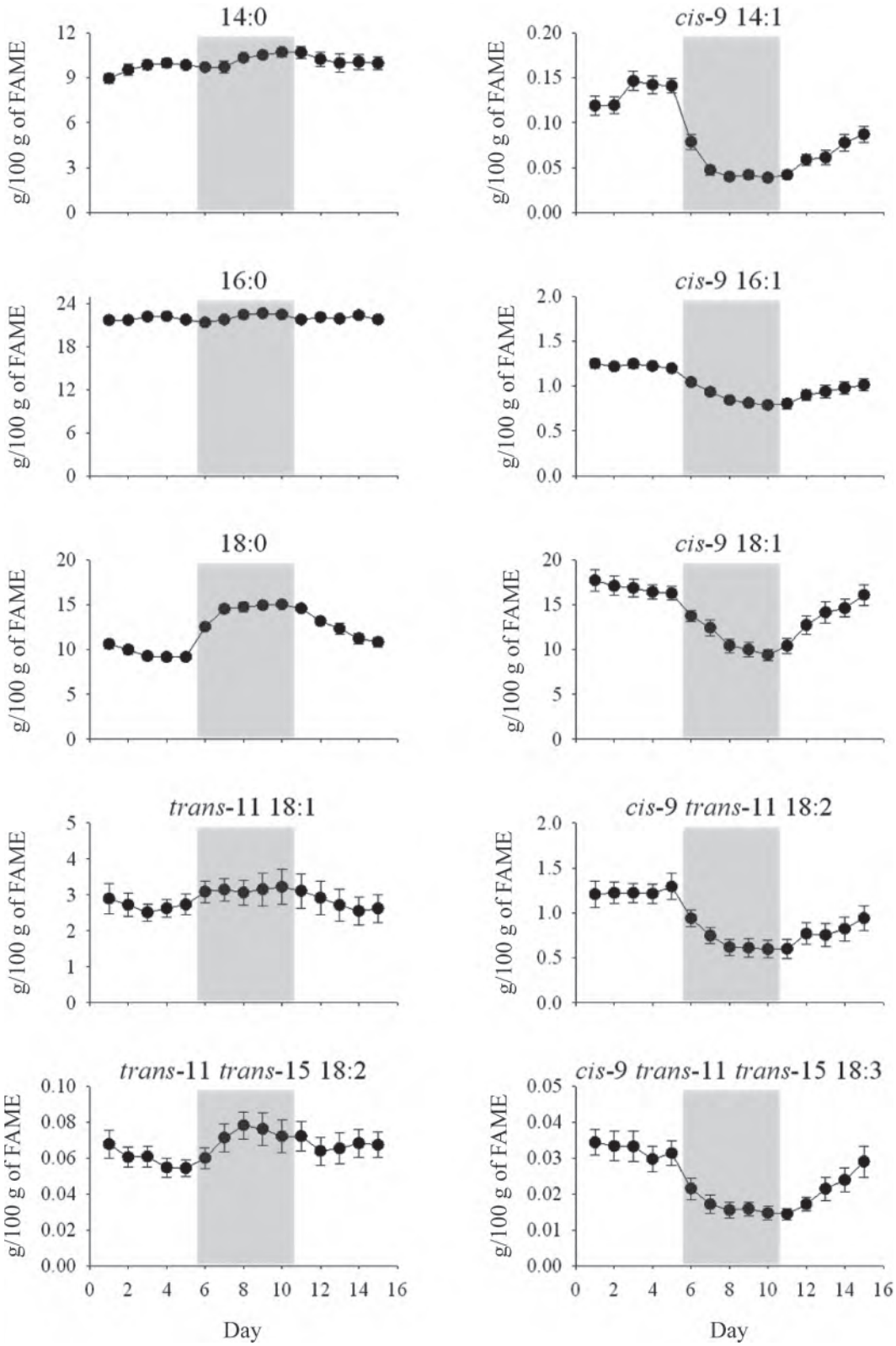

Figure 1. Temporal changes in milk 14:0, *cis*-9 14:1, 16:0, *cis*-9 16:1, 18:0, *cis*-9 18:1, *trans*-11 18:1, *cis*-9, *trans*-11 18:2, *trans*-11, *trans*-15 18:2, and *cis*-9, *trans*-11, *trans*-15 18:3 content [g/100 g of total FA methyl esters (FAME)] in dairy ewes before (d 1 to 5), during (d 6 to 10; gray shadow), and after (d 11 to 15) sterculic acid administration. Error bars represent the standard error of the mean (n = 6).

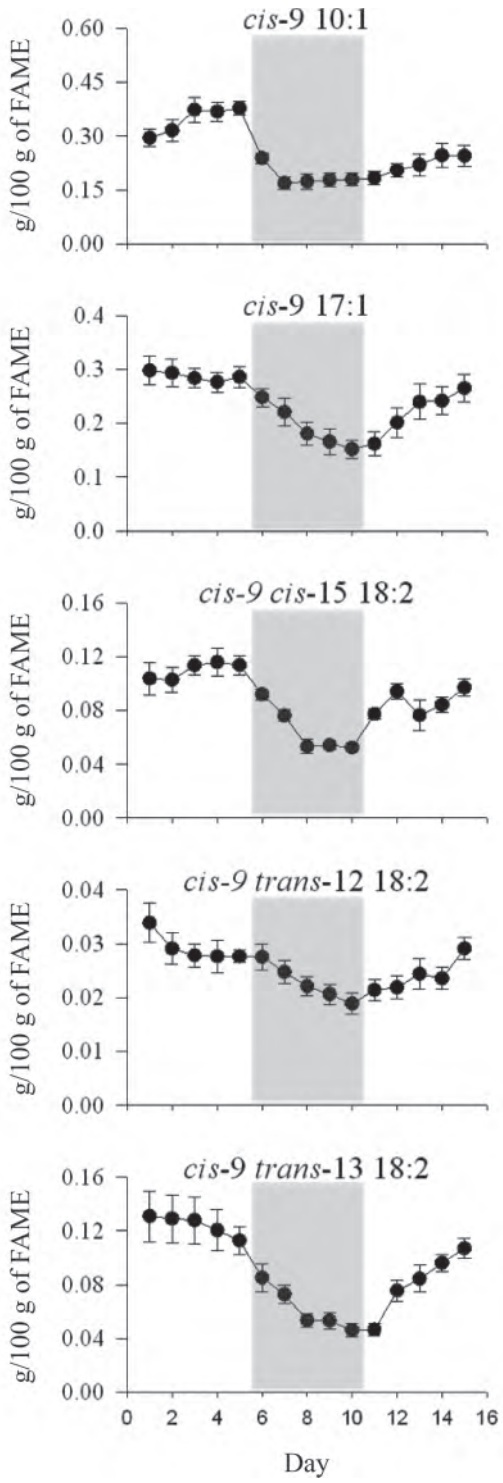

Figure 2. Temporal changes in milk *cis*-9 10:1, *cis*-9 17:1, *cis*-9, *cis*-15 18:2, *cis*-9, *trans*-12 18:2, and *cis*-9, *trans*-13 18:2 content [g/100 g of total FA methyl esters (FAME)] in dairy ewes before (d 1 to 5), during (d 6 to 10; gray shadow), and after (d 11 to 15) sterculic acid administration. Error bars represent the standard error of the mean (n = 6).

DISCUSSION

Previous research has shown that endogenous synthesis via Δ^9-desaturation is the major source of *cis*-9, *trans*-11 18:2 in cow and goat milk fat (Griinari et al., 2000; Corl et al., 2001; Bernard et al., 2010). The present study investigates the role of Δ^9-desaturase in mammary lipogenesis in the dairy sheep and provides the first estimates of endogenous synthesis of *cis*-9, *trans*-11 18:2 and *cis*-9 18:1 in ovine milk fat.

Sterculic acid administration did not affect milk yield, which is in agreement with previous studies using sterculic oil in lactating cows (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004). However, and also in accordance with results observed in experiments in caprines and bovines (Bickerstaffe and Johnson, 1972; Corl et al., 2001), slight reductions in milk fat content and yield were due to the treatment with sterculic acid. These reductions cannot be explained by changes in the proportion of FA synthesized de novo, but they are likely related to the decrease in the proportion of MUFA. The inhibition of Δ^9-desaturase has been reported to detrimentally affect milk fat synthesis, probably because the reduction in the content of *cis*-9 18:1 (and perhaps other Δ^9-desaturase products) influences the physical properties of milk fat by increasing its melting point (Chilliard et al., 2000). The persistence of enzyme inhibition over time might explain the persistence of the lower milk fat content in the posttreatment period. The DMI was also reduced in this period, but the experimental design did not allow us to attribute this effect to the treatment or to a period.

As expected, sterculic acid administration elicited a decrease in the concentration of Δ^9-desaturase products and a concurrent increase in many of its substrates because of the inhibitory effect of this cyclopropene FA on the activity of the enzyme (Jeffcoat and Pollard, 1977; Gomez et al., 2003). Inhibitory effects of sterculic acid are probably mediated via direct reductions in enzyme activity rather than by lowered Δ^9-desaturase gene expression or protein translation (Gomez et al., 2003; Palmquist et al., 2005). Beginning with *cis*-9 18:1, whose levels in the milk fat of grazing dairy sheep have been reported to range from 13 to up to 41% of total FA (Addis et al., 2005; Gómez-Cortés et al., 2009a; Buccioni et al., 2010), sterculic acid administration allowed us to estimate an endogenous synthesis of 63% in this ruminant species. This value is within the range calculated in cows (e.g., Enjalbert et al., 1998; Griinari et al., 2000; Shingfield et al., 2008; Taugbøl et al., 2008). One of the highest estimates in cows was calculated by abomasal infusion of sterculic acid in grazing animals (approximately 71%; Kay et al., 2004). In goats, the mean contribution of mammary desaturation to the

Table 4. Fatty acid profile of milk in dairy ewes before (pretreatment), during (treatment), and after (posttreatment) sterculic acid administration

FA, g/100 g of total FA methyl esters	Pretreatment	Treatment	Posttreatment	SED[1]	*P*-value[2]
SFA					
4:0	3.90[b]	4.29[a]	3.97[b]	0.051	<0.001
6:0	3.22	3.11	3.12	0.055	0.121
8:0	2.96	2.96	2.87	0.058	0.201
10:0	8.31	8.60	8.44	0.207	0.228
12:0	4.10[b]	4.34[a]	4.39[a]	0.080	0.003
13:0 *iso*	0.030[b]	0.032[ab]	0.035[a]	0.0014	0.009
13:0 *anteiso*	0.052[a]	0.029[c]	0.033[b]	0.0015	<0.001
13:0	0.20[a]	0.16[b]	0.16[b]	0.005	<0.001
14:0 *iso*	0.19	0.20	0.19	0.005	0.553
14:0	9.63[b]	10.19[a]	10.18[a]	0.163	0.001
15:0 *iso*	0.38[b]	0.43[a]	0.42[a]	0.012	<0.001
15:0 *anteiso*	0.78[a]	0.70[b]	0.69[b]	0.020	<0.001
15:0	1.42[a]	1.34[b]	1.34[b]	0.022	<0.001
16:0 *iso*	0.28[a]	0.27[b]	0.27[b]	0.006	0.018
16:0	21.89	22.11	21.96	0.226	0.602
17:0	0.79[b]	0.91[a]	0.93[a]	0.018	<0.001
18:0 *iso*	0.08[b]	0.08[b]	0.09[a]	0.004	0.003
18:0	9.49[c]	14.91[a]	12.84[b]	0.385	<0.001
19:0	0.06[b]	0.09[a]	0.09[a]	0.002	<0.001
20:0	0.19[b]	0.20[a]	0.21[a]	0.004	<0.001
21:0	0.070	0.071	0.075	0.0021	0.051
22:0	0.12	0.12	0.13	0.003	0.184
23:0	0.08[b]	0.08[b]	0.09[a]	0.003	<0.001
24:0	0.060[b]	0.057[b]	0.064[a]	0.0017	0.001
MUFA					
cis-9 10:1	0.35[a]	0.19[c]	0.22[b]	0.011	<0.001
cis-9 14:1	0.14[a]	0.04[c]	0.06[b]	0.005	<0.001
cis-9 15:1	0.059[c]	0.064[b]	0.076[a]	0.0017	<0.001
cis-7 16:1	0.47[b]	0.46[b]	0.51[a]	0.009	<0.001
cis-8 16:1	0.02[b]	0.02[b]	0.19[a]	0.001	<0.001
cis-9 16:1[3]	1.23[a]	0.88[b]	0.93[b]	0.030	<0.001
cis-10 16:1	0.04	0.03	0.04	0.002	0.662
cis-11 16:1	0.03	0.03	0.03	0.001	0.192
cis-12 16:1	0.035[b]	0.036[b]	0.038[a]	0.0011	0.004
cis-13 16:1	0.07[a]	0.02[c]	0.03[b]	0.005	<0.001
trans-9 16:1[4]	0.59[b]	0.62[a]	0.60[ab]	0.008	0.005
Other *trans* 16:1	0.11[b]	0.12[a]	0.12[a]	0.003	<0.001
cis-7 17:1	0.08[b]	0.09[a]	0.09[a]	0.002	<0.001
cis-9 17:1	0.29[a]	0.19[c]	0.22[b]	0.012	<0.001
cis-9 18:1	16.71[a]	9.98[c]	12.85[b]	0.465	<0.001
cis-11 18:1	0.28[c]	0.33[a]	0.30[b]	0.009	<0.001
cis-12 18:1	0.12	0.12	0.12	0.004	0.673
cis-13 18:1	0.05[b]	0.05[b]	0.06[a]	0.002	0.004
cis-15 18:1	0.12[c]	0.13[b]	0.16[a]	0.005	<0.001
cis-16 18:1	0.03[b]	0.04[a]	0.04[a]	0.002	<0.001
trans 6+7+8 18:1	0.21[b]	0.25[a]	0.25[a]	0.005	<0.001
trans-9 18:1	0.20[a]	0.17[b]	0.17[b]	0.004	<0.001
trans-10 18:1	0.27	0.27	0.25	0.011	0.360
trans-11 18:1	2.70[b]	3.13[a]	2.78[b]	0.111	<0.001
trans-12 18:1	0.19[b]	0.24[a]	0.25[a]	0.007	<0.001
trans-15 18:1	0.11[b]	0.18[a]	0.18[a]	0.006	<0.001
trans-16 18:1[5]	0.39[b]	0.40[b]	0.43[a]	0.009	<0.001
cis-11 20:1	0.02[b]	0.03[a]	0.03[a]	0.001	0.007
24:1	0.03[a]	0.03[a]	0.02[b]	0.001	<0.001
Nonconjugated 18:2					
cis-9, *cis*-12 18:2	1.41[c]	1.58[b]	1.69[a]	0.049	<0.001
cis-9, *trans*-12 18:2	0.03[a]	0.02[b]	0.02[b]	0.001	<0.001
cis-9, *cis*-15 18:2	0.11[a]	0.07[c]	0.09[b]	0.004	<0.001
cis-9, *trans*-13 18:2	0.12[a]	0.06[c]	0.08[b]	0.006	<0.001
trans-8, *cis*-13 18:2	0.09[b]	0.14[a]	0.09[b]	0.004	<0.001
trans-9, *cis*-12 18:2	0.04	0.05	0.04	0.001	0.376
trans-9, *trans*-12 18:2	0.06[a]	0.03[c]	0.04[b]	0.003	<0.001
trans-11, *cis*-15 18:2	0.34	0.36	0.35	0.013	0.269
trans-11, *trans*-15 18:2	0.06[b]	0.07[a]	0.07[a]	0.002	<0.001

Continued

Table 4 (Continued). Fatty acid profile of milk in dairy ewes before (pretreatment), during (treatment), and after (posttreatment) sterculic acid administration

FA, g/100 g of total FA methyl esters	Pretreatment	Treatment	Posttreatment	SED[1]	*P*-value[2]
Conjugated 18:2					
cis-9, *trans*-11 18:2	1.23[a]	0.70[c]	0.78[b]	0.043	<0.001
trans-9, *cis*-11 18:2	0.008[a]	0.005[c]	0.006[b]	0.0004	<0.001
trans-9, *trans*-11 18:2	0.008[a]	0.006[b]	0.006[b]	0.0004	<0.001
trans-10, *cis*-12 18:2	0.003[a]	0.002[b]	0.003[a]	0.0002	<0.001
trans-11, *cis*-13 18:2	0.12	0.12	0.12	0.008	0.794
trans-11, *trans*-13 18:2	0.09[b]	0.11[a]	0.11[a]	0.004	<0.001
trans-12, *trans*-14 18:2	0.01	0.01	0.01	0.001	0.347
trans-13, *trans*-15 18:2	0.01	0.01	0.01	0.001	0.652
Other PUFA					
cis-6, *cis*-9, *cis*-12 18:3	0.03[b]	0.03[b]	0.04[a]	0.001	<0.001
cis-9, *cis*-12, *cis*-15 18:3	1.42[c]	1.59[b]	1.87[a]	0.056	<0.001
cis-9, *trans*-11, *cis*-15 18:3	0.23	0.22	0.20	0.016	0.131
cis-9, *trans*-11, *trans*-15 18:3	0.032[a]	0.017[c]	0.021[b]	0.0013	<0.001
cis-11, *cis*-14 20:2	0.012[c]	0.014[b]	0.016[a]	0.0006	<0.001
cis-8, *cis*-11, *cis*-14 20:3	0.01[b]	0.01[b]	0.02[a]	0.001	0.001
cis-11, *cis*-14, *cis*-17 20:3	0.014[c]	0.016[b]	0.018[a]	0.0005	<0.001
cis-5, *cis*-8, *cis*-11, *cis*-14 20:4	0.140[a]	0.128[c]	0.137[b]	0.0034	0.002
cis-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5	0.09	0.08	0.09	0.004	0.058
cis-13, *cis*-16 22:2	0.15[a]	0.13[b]	0.12[b]	0.004	<0.001
cis-7, *cis*-10, *cis*-13, *cis*-16 22:4	0.03[a]	0.02[b]	0.03[a]	0.001	0.026
cis-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:5	0.17	0.16	0.16	0.005	0.455
cis-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:6	0.06	0.05	0.05	0.002	0.273

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

[1]SED = SE of the difference.

[2]Probability of significant differences between periods.

[3]Coelutes with 17:0 *anteiso*.

[4]Coelutes with 17:0 *iso*.

[5]Coelutes with *cis*-14 18:1.

synthesis of oleic acid was estimated at approximately 82% (Annison et al., 1967).

Regarding *cis*-9, *trans*-11 18:2, which is the most abundant CLA isomer in sheep milk fat and represents between 1.1 and 2.4% of total FA in grazing dairy ewes (Addis et al., 2005; Cruz-Hernandez et al., 2006; Gómez-Cortés et al., 2009a), its endogenous synthesis was estimated at 74%. This value is similar to the estimate calculated by Bernard et al. (2010) in dairy goats (63 to 73%) and within the range of 64 to 97% reported in cows (e.g., Palmquist et al., 2005; Mosley et al., 2006; Glasser et al., 2008). It is interesting that in a study in lactating cows fed fresh pasture and receiving an abomasal infusion of sterculic oil, the estimated value was higher than that observed in this study (91%; Kay et al., 2004). The authors attributed the high endogenous synthesis of milk rumenic acid to the elevated supply of *trans*-11 18:1 coming from ruminal biohydrogenation of α-linolenic acid, the most abundant FA in pasture. In sheep, the results from the present study not only show the expected relationship between rumenic and vaccenic acids (Griinari et al., 2000), but also suggest that mammary desaturation of *trans*-11 18:1 is not as elevated as in the cow. Therefore, given the high contents of rumenic acid in ovine milk (Park et al., 2007; Tsiplakou et al., 2009; Buccioni et al., 2010), it might be hypothesized that a greater portion of rumenic acid is coming from duodenal flow in sheep compared with that in cows. Consistent with this hypothesis, a comparison of data from studies on the FA profile of ruminal digesta showed that the content of rumenic acid is less abundant in cows (approximately 0.1%; AbuGhazaleh et al., 2002; Lock and Garnsworthy, 2002; Loor et al., 2004) than in ewes (approximately 0.2 to 0.3%; Toral et al., 2010, 2012).

Sterculic acid administration caused a decrease in the milk fat content of several Δ^9-desaturase products, such as *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 17:1, *cis*-9, *trans*-12 18:2, and *cis*-9, *trans*-13 18:2, reported to come from Δ^9-desaturation of 10:0, 14:0, 16:0, 17:0, *trans*-12 18:1, and *trans*-13 18:1, respectively (Mahfouz et al., 1980; Mosley and McGuire, 2007; Shingfield et al., 2007). A similar trend was observed for *cis*-9, *cis*-15 18:2 and *cis*-9, *trans*-11, *trans*-15 18:3, but they have not been described previously as Δ^9-desaturase products. However, the concurrent increases in *cis*-15 18:1 and *trans*-11, *trans*-15 18:2 during the treatment with sterculic acid allowed us to speculate that they might be 2 putative products coming from Δ^9-desaturation. To the knowledge of the authors, the isomer *cis*-9, *trans*-11, *trans*-15 18:3 has not been described to date in rumen effluent, plasma, or the products of in vitro

rumen metabolism of α-linolenic acid. The hypothesis of mammary Δ^9-desaturation of *trans*-11, *trans*-15 18:2 does not preclude the existence of a rumen biohydrogenation pathway involving *cis*-9, *trans*-11, *trans*-15 18:3, *trans*-11, *trans*-15 18:2, and *trans*-15 18:1, as postulated by Gómez-Cortés et al. (2009b), but it would imply a relevant role of the mammary gland in *cis*-9, *trans*-11, *trans*-15 18:3 synthesis.

In contrast, the ratio of *cis*-9, *trans*-11, *cis*-15 18:3 to *trans*-11, *cis*-15 18:2 plus *cis*-9, *trans*-11, *cis*-15 18:3 (also belonging to the α-linolenic acid biohydrogenation pathways) calculated from our data does not support the view that mammary Δ^9-desaturation of *trans*-11, *cis*-15 18:2 was the main source of *cis*-9, *trans*-11, *cis*-15 18:3 because its ratios in the pretreatment and treatment periods were not significantly different (Table 3). In fact, the group of isomers *cis*-9, *trans*-11, *cis*-15 18:3, *trans*-11, *cis*-15 18:2, and *cis*-15 18:1 has been found in the plasma of cows fed linseed (Akraim et al., 2007), which would indicate that they are predominantly originated in the rumen. Furthermore, Gómez-Cortés et al. (2009b) observed a higher ratio of *cis*-9, *trans*-11, *cis*-15 18:3 to *trans*-11, *cis*-15 18:2 plus *cis*-9, *trans*-11, *cis*-15 18:3 in ewes fed a control diet than in ewes fed an α-linolenic acid-enriched diet (0.57 vs. 0.09), which would also support the view that rumen biohydrogenation of α-linolenic acid was the main source of *cis*-9, *trans*-11, *cis*-15 18:3.

The decrease in *cis*-9 14:1 and *cis*-9 16:1 was expected to be accompanied by a concurrent increase in their corresponding substrates (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004). However, as previously reported by Corl et al. (2001), no change was observed in 16:0 concentration, which would suggest that a low proportion of this FA is subjected to desaturation. According to Corl et al. (2001, 2002), the increase in *trans*-6+7+8 18:1 might reflect the use of *trans*-7 18:1 to synthesize *trans*-7, *cis*-9 18:2.

It was surprising that sterculic acid administration seemed to modify odd- and branched-chain FA. Nevertheless, temporal variations in these FA might be independent of the treatment because no evidence exists in the literature that sterculic acid affects ruminal metabolism, and branched-chain FA are known to be largely derived from bacteria leaving the rumen (Vlaeminck et al., 2006). On the other hand, part of these FA may be synthesized de novo in the mammary gland in lactating ewes (Vlaeminck et al., 2006); therefore, further research would be necessary to clarify this behavior.

Finally, sterculic acid administration affected other FA that were not correlated with the Δ^9-desaturase system. Thus, the percentage of linoleic and α-linolenic acids increased in the sterculic treatment, in agreement with results observed in cows when Δ^9-desaturase was inhibited with cobalt (Taugbøl et al., 2010). This effect was attributed to cobalt interfering not only with Δ^9-desaturase but also with other Δ-desaturase enzymes (e.g., Δ^5 and Δ^6) that would convert, for example, α-linolenic acid into *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5 (Taugbøl et al., 2010). Even though the mode of action of sterculic acid is not completely understood, Cao et al. (1993) demonstrated that cyclopropene FA inhibit Δ^5- and Δ^6-desaturase systems in rat liver microsomes. Thus, even though some authors found no changes in 18:2n-6 and 18:3n-3 when they infused sterculic oil in cows (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004) and only little evidence of Δ^6-desaturase exists in the mammary gland (Bionaz and Loor, 2008), it should not be ruled out that an inhibition of this enzyme in other tissues might explain, at least in part, their increase in this study. In addition, an inhibition of Δ^5-desaturase would account for the reduction in 20:4n-6 and the tendency in 20:5n-3.

CONCLUSIONS

Administration of sterculic acid to lactating ewes resulted in a 70% inhibition of Δ^9-desaturase in the mammary gland that persisted partially over time. Similar to previous findings in the cow and goat, endogenous synthesis was the major source of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2, accounting for 63 and 74%, respectively, of its content in ovine milk fat.

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