J. Dairy Sci. 88:1478–1489
© American Dairy Science Association, 2005.

Mammary Lipid Metabolism and Milk Fatty Acid Secretion in Alpine Goats Fed Vegetable Lipids

L. Bernard,[1] J. Rouel,[1] C. Leroux,[1] A. Ferlay,[1] Y. Faulconnier,[1] P. Legrand,[2] and Y. Chilliard[1]
[1]Unité de Recherche sur les Herbivores, Institut National de la Recherche Agronomique (INRA), Theix, 63 122 St. Genès-Champanelle, France
[2]Laboratoire de Biochimie, Ecole Nationale Supérieure Agronomique, 65 rue de Saint-Brieuc, 35 000 Rennes, France

ABSTRACT

Fourteen Alpine goats at midlactation were fed a diet of hay and concentrate (55:45), without (control) or with formaldehyde-treated linseed (FLS) or oleic sunflower oil (OSO) at 11.2 or 3.5% of dry matter intake, respectively, in a 3 × 3 Latin Square design with three 3-wk periods. Milk yield was lower in goats fed FLS than control or OSO (2.13 vs. 2.32 kg/d). Milk fat content was higher with FLS or OSO than control (40.8 vs. 33.8 g/kg). Formaldehyde-treated linseed and OSO caused a significant decrease (23 and 18%, respectively) of C10 to C17 fatty acids secretion compared with control. The secretion of *cis*-9 C18:1 and *cis*-9, *trans*-11 C18:2 were increased 1.44- and 1.54-fold for FLS and 1.78- and 1.36-fold for OSO, compared with control. The C18:3 (n-3) secretion was increased 2.61-fold with FLS compared with control. Milk *cis*-9 C14:1/C14:0, *cis*-9 C16:1/C16:0, and *cis*-9 C18:1/C18:0 ratios decreased with the supplemented diets compared with control. Mammary stearoyl-CoA desaturase mRNA and activity were decreased by the lipid supplements, whereas no significant change was observed for acetyl-CoA carboxylase and fatty acid synthase. The activities of glucose-6-phosphate dehydrogenase, malic enzyme, and glycerol-3-phosphate dehydrogenase were not affected by the lipid supplements. Mammary lipoprotein lipase mRNA increased with OSO, whereas lipoprotein lipase activity tended to decrease with FLS compared with control. Milk lipoprotein lipase activity sharply decreased with lipid supplement (by 59 and 71%, for FLS and OSO, respectively). The changes in milk fatty acid profile due to FLS and OSO supplements were partly related to changes in the levels of mammary enzyme activities or mRNA.
(**Key words:** lactating goat, lipid supplement, lipogenic enzyme, milk fatty acids)

Abbreviation key: ACC = acetyl-CoA carboxylase, **FA** = fatty acid, **FAS** = fatty acid synthase, **FLS** = formaldehyde-treated linseed, **G3PDH** = glycerol-3-phosphate dehydrogenase, **G6PDH** = glucose-6-phosphate dehydrogenase, **LPL** = lipoprotein lipase, **ME** = malic enzyme, **OSO** = oleic sunflower oil, **PUFA** = polyunsaturated fatty acids, **SCD** = stearoyl-CoA desaturase.

INTRODUCTION

Milk fat is an important component of the nutritional quality of goat dairy products. For example, some fatty acids (**FA**) found in milk triacylglycerol have been shown to exert positive effects on human health such as, for oleic and linolenic acid, a cardioprotective effect through a direct vascular antiatherogenic action (Massaro et al., 1999). Furthermore, goat milk fat content and composition can be extensively modified by genetic and physiological factors as well as by nutritional factors (Chilliard et al., 2003a). Among nutritional factors, fat supplementation of the diet is an efficient means to modify milk FA composition in lactating ruminants (Palmquist et al., 1993; Chilliard et al., 2000), which could be used to improve the nutritional quality of milk fat. Milk fatty acids have 2 main origins: they are synthesized de novo in the mammary gland or extracted from the arterial blood. These processes involve numerous mammary enzymes, including lipoprotein lipase (**LPL**), acetyl-CoA carboxylase (**ACC**), fatty acid synthase (**FAS**), and stearoyl-CoA desaturase (**SCD**). Particular attention has been focused on SCD in the last decade because of its implication in the synthesis of monounsaturated fatty acids [mainly oleic (*cis*-9 C18:1) and palmitoleic (*cis*-9 C16:1) acids] and the major conjugated linoleic acid isomer (*cis*-9, *trans*-11 C18:2; Corl et al., 2001) found in ruminant milk fat. Differences in mammary gland levels of SCD may explain, in part, the substantial variations of these fatty acids in milk fat.

Received May 11, 2004.
Accepted December 12, 2004.
Corresponding author: Yves Chilliard; e-mail: Yves.Chilliard@clermont.inra.fr.

In goats, little data is available on the effect of nutritional factors on mammary and adipose tissue metabolism and in particular on the expression of genes for the key lipogenic enzymes. Thus, to better understand the mammary mechanisms underlying milk fat composition in response to different dietary factors, we investigated the effects of 2 dietary lipid supplements on mammary and adipose tissue metabolism, and milk fatty acid secretion, in lactating goats. These supplements were chosen to provide the mammary gland with C18 FA differing in their degree of unsaturation, to study their potential effect on mammary metabolism and particularly on SCD mRNA and enzyme activity because linolenic, linoleic, and oleic acids, compared with stearic acid, have been reported to inhibit SCD activity in goat mammary glands in vitro (Bickerstaffe and Annison, 1970). One of these supplements is rich in oleic acid (oleic sunflower oil, **OSO**) unprotected from ruminal biohydrogenation to favor the yield of stearic acid, whereas the other is rich in linolenic fatty acid in a protected form to partly escape biohydrogenation in the rumen (formaldehyde-treated linseed, **FLS**).

MATERIALS AND METHODS

Animals and Diets

Fourteen Alpine goats in midlactation (at 12 ± 1 wk of lactation at the beginning of the experiment) were fed 3 diets in a 3 × 3 Latin Square design (with 5, 5, or 4 animals per group, respectively). The goats were chosen based on the homogeneity of their milk yield, number of lactations, and taking into account their genotype at the *αS1* casein locus because of its reported effect on milk traits (Grosclaude et al., 1994). Each period lasted 3 wk, which included a 2-wk adjustment period followed by a sampling period. The diet consisted of orchardgrass hay distributed ad libitum and a mixture of concentrate (determined according to the initial milk yield) in a 50:50 ratio. This hay-based diet (Table 1) included a lipid supplement of FLS (11.2% DMI; linseed was ground and treated at the rate of 3000 ppm of formaldehyde, by INZO, Chateau-Thierry, France) or OSO (3.6% DMI), or no lipid supplement (control). The quantity (g/d) of the main fatty acids provided by the diet is presented in Table 1. Goats were milked at 0800 and 1600 h. Goats were housed in individual stalls, had free access to water, and were fed twice a day, just after milking; the amounts fed and refused were recorded daily. The energy balance (kJ/d) was calculated as the difference between the net energy intake and the required energy calculated from milk yield, milk composition, and BW values. Goat weight was recorded at the start of the trial and at the end of each experimental period. The goats were cared for and handled in compliance with the guidelines of the INRA Animal Care Committee.

Table 1. Ingredients and chemical composition of diets unsupplemented (control) or supplemented with 3.6% lipid of formaldehyde-treated linseed (FLS) or oleic sunflower oil (OSO).

	Control	FLS	OSO
Ingredients (% of DM)			
Orchardgrass hay	54.0	52.1	55.4
Formaldehyde-treated linseed[1]	0.0	11.2	0.0
Oleic sunflower oil[2]	0.0	0.0	3.6
Concentrate mixture			
Rolled barley	26.5	17.0	19.7
Dehydrated sugar beet pulp	8.2	8.2	7.4
Pelleted dehydrated lucerne	5.6	4.9	4.8
Soybean meal	5.6	6.6	9.1
Mineral-vitamin mix[3]	0.015	0.015	0.015
Chemical composition (% of DM)			
OM	92.3	92.5	88.8
CP	15.0	16.7	15.7
NDF	46.5	45.5	46.0
ADF	24.2	24.5	24.0
Crude fiber	21.8	21.8	21.7
Ether extract	2.0	5.7	5.5
Intake of fatty acid (g/d)			
C16:0	5.44	8.99	8.00
C18:0	0.83	3.01	3.37
C18:1c9	5.96	14.98	57.57
C18:2 (n-6)	9.61	19.29	16.00
C18:3 (n-3)	4.14	46.13	4.03

[1]Formaldehyde-treated linseed was composed of (g/100 g of total fatty acids): C16:0, 6.4; C18:0, 3.2; *cis*-9 C18:1, 13.9; C18:2 (n-6), 15.9; C18:3 (n-3), 58.8.

[2]Oleic sunflower oil was composed of (g/100 g of total fatty acids): C16:0, 4.6; C18:0, 3.8; *cis*-9 C18:1, 77.7; C18:2 (n-6), 10.6; C18:3 (n-3), 0.4.

[3]Mineral-vitamin mix provided (g/kg of premix): Ca, 150; P, 100; Mg, 20; Na, 320; Zn, 6; Mn, 4; Vitamin B_1, 400; in (IU/kg of premix): retinyl acetate, 550,000; cholecalciferol, 40,000; DL-α-tocopherol acetate, 400.

Sampling and Analysis

Milk yield was recorded 3 d/wk (from 6 consecutive milkings). Milk samples were collected on the last week of each experimental period on d 18 and 19 (from 4 consecutive milkings) for analysis of milk fat and protein contents (AOAC, 1997; CILAL, Theix, France). Two milk samples (a.m. and p.m. milkings) were collected on d 19 of each experimental period, and stored at –20°C until the determination of FA composition on a combined sample. For LPL assay, one milk sample (a.m. milking only) was collected and stored at –20°C until analysis.

At the end of the experiment, the goats were slaughtered just after milking and feeding. Milking was completed to eliminate most of the milk contained in the glands. After death, mammary and perirenal adipose

tissues samples were collected under sterile conditions. Tissue samples were immediately frozen in liquid nitrogen and stored at –80°C until RNA extraction and enzyme assays. For SCD, collected mammary samples were immediately assayed for enzyme activity. For perirenal adipose tissue, a second sample was placed at 37°C, immediately after slaughtering, for adipocyte volume determination (Bonnet et al., 2000).

Plasma Measurements

Blood samples were collected in tubes containing EDTA (Venoject, C.M.L., Nemours, France) from the jugular vein at 0730 h on d 19 of each experimental period for the determination of plasma insulin (Insulin CT kit; CIS Bio International, Gif-sur-Yvette, France) and metabolites. Plasma levels of glucose, NEFA, BHBA, and urea were determined as described by Ferlay and Chilliard (1999) with an ELAN auto-analyzer (Merck-Clévenot SA, Nogent-sur-Marne, France) and by spectrophotometric enzymatic assays using specific kits (Glucose S-system 10, Merck-Clévenot SA; NEFA C WAKO, Unipath SA, Dardilly, France; Urea SMT, Merck, Germany).

Analysis of Milk FA

Fatty acids in orchardgrass hay and the concentrate mixture were extracted according to the method of Folch et al. (1957) and then methylated as described by Ferlay et al. (1993). Fatty acids from 3 mL of lyophilized milk were extracted in 10 mL of hexane:diethylether (50:50, vol/vol), 1 mL of saturated NaCl solution, and 1 mL of ethanol mixture by homogenization, and centrifugation at 1000 rpm for 10 min at 4°C, followed by a 3-fold extraction in 5 mL of hexane:diethylether (50:50, vol/vol) and evaporation. Fatty acids were methylated by the method of Glass (1971) modified by J. L. Sébédio (personal communication, 2000), by addition of 100 μL of sodium methanoleate (1 *M*) at room temperature for 10 min followed by 500 μL of 14% boron trifluoride in methanol for 10 min. For feedstuffs and milk samples, fatty acid methyl esters were recovered in 1 mL of hexane. For feedstuffs, C17:0 (Sigma, Saint-Quentin Fallavier, France) was used as an internal standard. Samples were injected by auto-sampler into a Trace-GC 2000 Series gas chromatograph equipped with a flame ionization detector (Thermo Finnigan, Les Ulis, France). Methyl esters from all samples were separated on a 100-m × 0.25-mm i.d. fused silica capillary column (CP-Sil 88, Chrompack, Middelburg, the Netherlands). A custom preparation made with individual *trans*-C18:1, *cis*-C18:1, nonconjugated C18:2 isomers, and conjugated linoleic acid isomer methyl esters (Sigma) was used for their identification. A butter reference standard (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain (4:0 to 10:0) fatty acids.

For feedstuff and milk FA analysis, the injector temperature was maintained at 250°C and the detector temperature was maintained at 255°C. The initial oven temperature was held at 70°C for 1 min, increased by 5°C/min to 100°C (held for 2 min), then increased by 10°C/min to 175°C (held for 40 min), and increased by 5°C/min to a final temperature of 225°C (held for 15 min). Hydrogen was the carrier gas. Injector pressure was held constant at 23 psi.

Enzyme Assays

Lipoprotein lipase (EC 3.1.1.34) activity was measured in perirenal adipose tissue and mammary gland using an artificial emulsion containing [^{3}H]-triolein after a detergent (deoxycholate-Nonidet P40; Sigma) extraction procedure (Faulconnier et al., 1994). Activity of LPL in the milk samples was measured using the same technique except that the first step of extraction was omitted.

Activities of FAS (EC 2.3.1.85), glycerol-3-phosphate dehydrogenase (**G3PDH**, EC 1.1.1.8), glucose-6-phosphate dehydrogenase (**G6PDH**, EC 1.1.1.49), and malic enzyme (**ME**, EC 1.1.1.40) were measured spectrophotometrically in perirenal adipose tissue and mammary gland as described previously (Chilliard et al., 1991).

Acetyl-CoA carboxylase (EC 6.4.1.2) activity was determined for mammary tissue and assayed by the $H^{14}CO3^-$ fixation method (Chakrabarty and Leveille, 1969). The assay was adapted to mammary tissue to obtain a linear relationship of the activity with incubation time and protein content. The enzyme activity was calculated as nanomoles of $^{14}CO_2$ incorporated per minute per milligram of soluble protein. In our conditions, it was checked that there was no significant interference in the assay by other carboxylase enzymes by measuring the level of incorporation of $^{14}CO_2$ into the soluble fraction in the absence of citrate (which represented 1.5 to 2.2% of the total activity).

Stearoyl-CoA desaturase (EC 1.14.99.5) activity was determined for mammary tissue and was assayed by the method of conversion of [^{14}C]-stearic acid into [^{14}C]-oleic acid according to Legrand et al. (1997). The assay was adapted to mammary tissue to get linear activity with incubation time and protein content. The enzyme activity was calculated as nanomoles of stearic acid converted to oleic acid per minute per milligram of protein.

Protein content of the homogenates was determined by a modified Lowry method (Bensadoun and Weinstein, 1976) using BSA as the standard.

RNA Extraction

Mammary tissue total RNA was prepared using guanidinium-thiocyanate extraction as described by Cathala et al. (1983). Integrity of RNA was verified by ethidium bromide staining following agarose gel electrophoresis, and the concentration was determined by spectrophotometric (260 nm) analyses.

Analysis of mRNA using Real-Time Reverse-Transcription PCR

Levels of SCD, ACC, FAS, and LPL mRNA in mammary tissues were quantified by real-time quantitative reverse-transcription PCR. Single-strand cDNA was obtained from total RNA by reverse transcription as previously described (Bernard et al., 2001). The absence of genomic DNA was controlled by PCR using a pair of primers from β-actin, including a small intron within the gene (I. Hue, personal communication, 2000) that would yield PCR products of either 400 or 200 bp when starting from genomic DNA or cDNA, respectively.

Polymerase chain reaction was carried out on a LightCycler system (Roche Molecular Biochemicals, Indianapolis, IN), using the fluorescent probe Taqman methodology. Specific primers (Genosys Biotechnology, UK; concentration adjusted to 10 pmol/μL) and Taqman probes (Applied Biosystems, Warrington, UK; concentration adjusted to 10 pmol/μL) were as follows: for SCD, 5′-TGCTGACAACTTATCTGGATGC-3′(sense), 5′-AAGGAATCCTGCAAACAGCTA-3′ (antisense), and 5′-CCAGAGCCTGCAGAAGTGGCTGGTATAA-3′(Taqman probe); for ACC, 5′-CATGGAAATGTACGCGGACC-3′(sense), 5′-GGTGGTAGATGGGAAGGAGGA-3′(antisense), and 5′-CGAGCGGAAGGAGCTGGAGAGCA-3′ (Taqman probe); for FAS, 5′-ACAGCCTCTTCCTGTTTGACG-3′(sense), 5′-CTCTGCACGATCAGCTCGAC-3′(antisense), and 5′-ATCTGGAGGCGCGTGTGGCAGCC-3′(Taqman probe); and for LPL, 5′-TTCAGAGGCTATTACTGGAAATCC-3′(sense), 5′-ATGTCAATCACAGCATTCATTCTACT-3′(antisense), and 5′-TTCCAGTGGTGCCGGAACACTCCTTC-3′(Taqman probe), and yielded fluorescent PCR products of 179, 230, 226, 186 bp for SCD, ACC, FAS, and LPL, respectively. To take into account RNA quality and quantity and cDNA synthesis, cyclophilin mRNA, a housekeeping gene, was quantified using primers and probe as described by Bonnet et al. (2000) yielding a 250-bp PCR product.

For each mRNA, the quantification was determined from a calibration curve prepared by amplifying a different copy number of a recombinant plasmid containing the same specific sequence of each gene as amplified as described for LPL and cyclophilin (Bonnet et al., 2000). The PCR carried out on the LightCycler system allowed amplification and detection of the fluorescence in a capillary tube. For LightCycler PCR, a master mixture of the following reaction components was prepared to the indicated final concentrations: 8.8 μL of water, 3.2 μL of $MgCl_2$ (5 mM), 0.8 μL of sense primer (0.4 μM), 0.8 μL of antisense primer (0.4 μM), 0.4 μL of the Taqman probe (0.2 μM), and 2 μL of LightCycler-FastStart DNA Master Hybridization Probes (Roche Applied Science, Meylan, France). Amplification and quantification were performed according to the manufacturer's instructions with conditions: 95°C for 10 s, 60°C for 30 s with a single fluorescence measurement repeated 40 times. Results were expressed as the mRNA copy number of each gene of interest relative to cyclophilin.

Statistical Analyses

Data from the 3 experimental periods were submitted to an ANOVA by the GLM procedure of SAS (SAS Institute, 2000) for a 3×3 Latin Square design. The model included effects of diet, period, and goat. Means were compared using the least square means procedure (SAS Institute, 2000) and the level of significance declared at $P < 0.05$.

Data from slaughter samples were tested for effects of nutritional treatments on mRNA quantification and enzyme activities using the nonparametric Wilcoxon U-test with differences considered significant when $P < 0.05$.

RESULTS

Diet Composition

The CP content and NDF and ADF concentrations of the diets were not different and averaged 15.8, 46, and 24.2% of DM, respectively (Table 1). The total lipid (ether extract) content of the control diet was 2% compared with 5.7 and 5.5% of DM, respectively, for the FLS and OSO diets. The amounts of individual fatty acids in each diet were primarily a function of their levels in feed ingredients. The major FA provided by the diets was linoleic (9.61 g/d), linolenic (46.13 g/d), and oleic (57.57 g/d) acids, respectively, for control, FLS, and OSO. The total C18 FA provided by the diets were respectively, 20.69, 84.01, and 81.68 g/d, for control, FLS, and OSO (Figure 1).

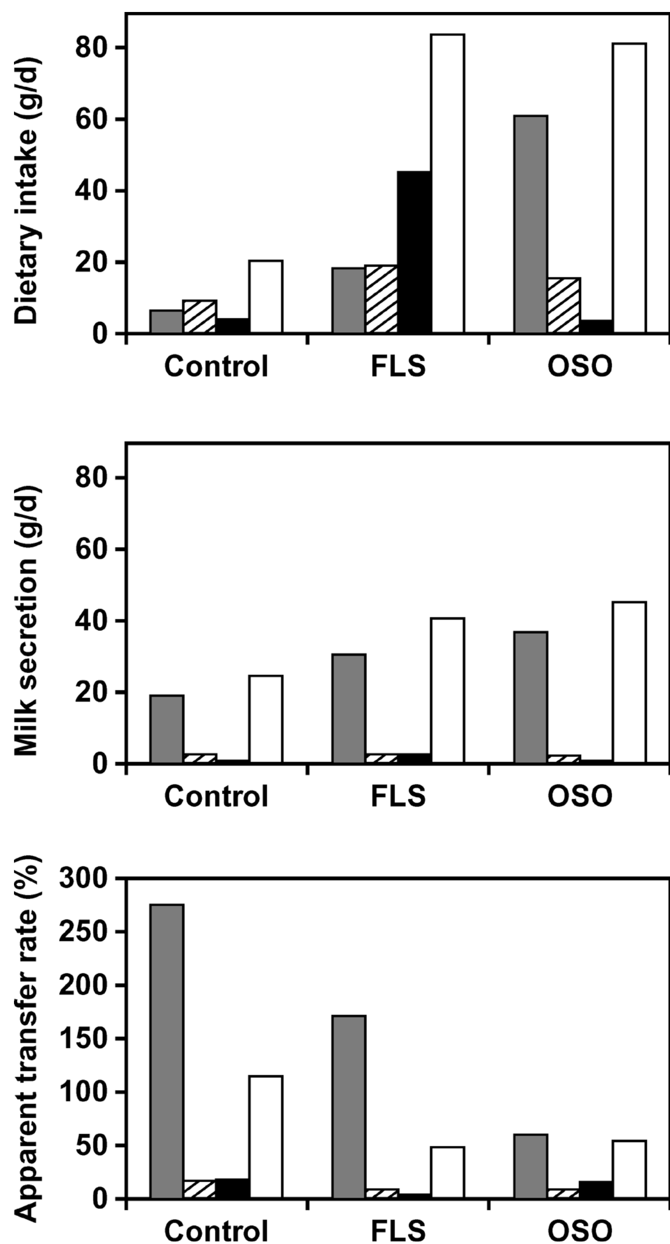

Figure 1. Intake (g/d), milk secretion (g/d), and apparent transfer rate [calculated as the ratio between the fatty acid secreted in milk and its level provided by the diet (%)] of C18 fatty acids: C18:0 + *cis*-9 C18:1 (gray bar), (n-6) C18:2 (hatched bar), (n-3) C18:3 (black bar) and the sum of C18 FAs (white bar), for control, formaldehyde-treated linseed (FLS), and oleic sunflower oil (OSO) diets fed to lactating goats.

DMI and Milk Production and Composition

Dry matter intake decreased slightly in response to both lipid supplements ($P < 0.05$). Formaldehyde-treated linseed significantly decreased (–0.24 kg/d) milk yield and significantly increased milk fat content (6.6 g/kg), fat yield (6.0 g/d), and milk protein content (1.2 g/kg; Table 2). Oleic sunflower oil caused an increase in the milk fat content (7.4 g/kg) and yield (13.0 g/d). The energy balance was positive and not significantly different between diets (Table 2), with a mean value of 760 kJ/d.

Table 2. Dry matter intake, milk yield, and milk composition of lactating Alpine goats fed a hay-based diet unsupplemented (control) or supplemented with 3.6% lipid of formaldehyde-treated linseed (FLS) or oleic sunflower oil (OSO).

Item	Control	FLS	OSO	SEM[1]
DM Intake (kg/d)	1.98[a]	1.85[b]	1.90[b]	0.02
Milk (kg/d)	2.37[a]	2.13[b]	2.27[a]	0.02
Fat (g/kg)	33.8[a]	40.4[b]	41.2[b]	3.5
Fat (g/d)	80[a]	86[b]	93[c]	1.07
Protein (g/kg)	32.2[a]	33.4[b]	32.2[a]	1.5
Protein (g/d)	75[a]	70[b]	73[ab]	0.77
Energy balance (KJ/d)	926	641	712	95

[a,b,c]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Standard error of least square means (n = 42).

Plasma Metabolite Concentrations

Glucose concentration was reduced ($P < 0.05$) with FLS compared with control diet. Glucose and insulin values were similar for OSO and control diets (Table 3). Nonesterified fatty acids, BHBA, and urea concentrations were similar for the 3 diets.

Adipose Tissue Lipid Metabolism

A significant ($P < 0.05$) increase in the activity of ME expressed per 10^6 adipocytes was observed for FLS compared with control diet (Table 4) and, to a lower extent, for OSO. No significant effect of the dietary treatments was observed on G6PDH, LPL, and FAS activities. For G3PDH, a tendency to increase the activity was observed with FLS ($P = 0.07$) and with OSO ($P = 0.11$) compared with control.

Table 3. Concentration of insulin and metabolites in plasma and lipoprotein lipase (LPL) activity in milk from lactating goats fed a hay-based diet supplemented or not (control) with 3.6% lipid of formaldehyde-treated linseed (FLS) or oleic sunflower oil (OSO).

Item	Control	FLS	OSO	SEM[1]
Insulin, μIU/mL	17.45[ab]	14.01[b]	19.05[a]	0.760
Glucose, m*M*	3.24[a]	3.09[b]	3.23[a]	0.03
NEFA, m*M*	0.107	0.155	0.138	0.012
BHBA, m*M*	0.288	0.269	0.270	0.006
Urea, m*M*	7.38	7.14	7.89	0.18
LPL, nmol/min per mL	170.2[a]	70.2[b]	48.6[b]	13.1

[a,b,c]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Standard error of least square means (n = 42).

Table 4. Activities[1] of lipoprotein lipase, fatty acid synthase, glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme, and glycerol-3-phosphate dehydrogenase (G3PDH) in the perirenal adipose tissue of lactating goats fed a hay-based diet supplemented or not (control) with 3.6% lipid of formaldehyde-treated linseed (FLS) or oleic sunflower oil (OSO).

Item	Control (n = 4)	FLS (n = 5)	OSO (n = 5)
Lipoprotein lipase	84.8 ± 35.6	135 ± 26.0	90.7 ± 8.63
Fatty acid synthase	37.4 ± 11.0	44.6 ± 5.8	59.7 ± 10.1
G6PDH	287 ± 64	341 ± 32	391 ± 75
Malic enzyme	36.1 ± 10.1[b]	77.1 ± 9.5[a]	79.4 ± 21.7[ab]
G3PDH	3020 ± 670	5030 ± 410	4900 ± 860

[a,b,c]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Values represent the mean ± SE of 4, 5, or 5 observations for Control, FLS, and OSO, respectively; values expressed as nmol/min per 10^6 adipocytes.

Milk LPL Activity

The LPL activity in milk decreased dramatically and significantly ($P < 0.05$) with OSO and FLS compared with control (Table 3).

Milk Fatty Acid Composition and Yield

Both FLS and OSO diets decreased ($P < 0.05$) the percentage of C6 to C17 saturated fatty acids in milk fat (Table 5) as well as the secretion of C10 to C17 saturated fatty acids (Table 6), compared with the control diet. For C17:0 percentage in milk fat, the decrease was more pronounced with OSO than with FLS diet and was similar for these diets when C17:0 was expressed as secretion. Both OSO and FLS diets led to a significant ($P < 0.05$) increase in the percentage and secretion of C18:0 in milk fat. The FLS and OSO diets reduced the C16:1 and C14:1 percentage and secretion in milk fat. Both OSO and FLS diets led to a significant increase of *cis*-9 C18:1 percentage and secretion (increasing in the following order: control < FLS < OSO) and FLS led to a significant increase of C18:3 (n-3) percentage and secretion (increasing in the following order: OSO ≤ control < FLS) due to the protection of these FA provided by the diet against ruminal biohydrogenation and to

Table 5. Composition of milk fatty acids from Alpine goats fed a hay-based diet unsupplemented (control) or supplemented with 3.6% lipid of formaldehyde-treated linseed (FLS) or oleic sunflower oil (OSO).

Item	Control	FLS	OSO	SEM[1]
	— g/100 g of total fatty acids —			
C4:0	2.64	2.74	2.68	0.04
C6:0	2.11[a]	1.94[b]	1.99[b]	0.02
C8:0	2.41[a]	2.16[b]	2.13[b]	0.02
C10:0	9.35[a]	7.29[b]	7.03[b]	0.09
C11:0	0.24[a]	0.16[b]	0.16[b]	0.01
C12:0	5.35[a]	3.34[b]	3.14[b]	0.06
C13:0	0.28[a]	0.16[b]	0.14[b]	0.01
C14:0	11.99[a]	8.86[b]	8.78[b]	0.11
C14:1 c9	0.24[a]	0.13[b]	0.13[b]	0.01
C15:0	1.29[a]	0.98[b]	0.91[b]	0.01
C16:0	27.47[a]	19.79[b]	19.09[b]	0.24
C16:1 c9	0.76[a]	0.43[b]	0.44[b]	0.01
C17:0	0.90[a]	0.66[b]	0.58[c]	0.01
C18:0	6.92[a]	13.53[b]	14.42[b]	0.24
Other C18:1 t[2]	0.61[a]	1.13[b]	2.42[c]	0.04
C18:1 t11 (+ t10)	1.08[a]	2.09[c]	1.83[b]	0.05
C18:1 c9	16.41[a]	21.84[b]	25.24[c]	0.26
Other C18:1 c[3]	0.81[a]	1.48[c]	1.16[b]	0.03
C18:2 c9c12	1.99[a]	1.92[b]	1.40[c]	0.01
C18:2 c9t11	0.77[a]	1.09[c]	0.90[b]	0.02
C18:3 c9c12c15	0.96[a]	2.29[c]	0.69[b]	0.04
C20:0	0.22[a]	0.23[a]	0.28[b]	0.01

[a,b,c]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Standard error of least square means (n = 42).

[2]Other C18:1t = t4, t5, t6, t7, t8, and t9.

[3]Other C18:1c = c11, c12, c13, c14 (including the t16), and c15 (including the t17).

Table 6. Secretion (g/d) of milk fatty acids from Alpine goats (n = 42) fed a hay-based diet unsupplemented (control) or supplemented with 3.6% lipid of formaldehyde treated linseed (FLS) or oleic sunflower oil (OSO).

Item	Control	FLS	OSO	SEM[1]
C4:0	2.09[a]	2.34[b]	2.49[b]	0.05
C6:0	1.70	1.65	1.84	0.04
C8:0	1.93	1.85	1.99	0.03
C10:0	7.50[a]	6.21[b]	6.61[b]	0.12
C11:0	0.19[a]	0.13[b]	0.15[b]	0.01
C12:0	4.21[a]	2.82[b]	2.94[b]	0.06
C13:0	0.22[a]	0.14[b]	0.13[b]	0.01
C14:0	9.53[a]	7.50[b]	8.18[b]	0.14
C14:1 c9	0.19[a]	0.11[b]	0.12[b]	0.01
C15:0	1.01[a]	0.83[b]	0.84[b]	0.02
C16:0	22.02[a]	16.84[b]	17.86[b]	0.33
C16:1 c9	0.60[a]	0.36[c]	0.42[b]	0.01
C17:0	0.72[a]	0.56[b]	0.54[b]	0.01
C18:0	5.60[a]	11.91[b]	13.35[b]	0.29
Other C18:1 t[2]	0.46[a]	0.94[b]	2.20[c]	0.05
C18:1 t11 (+ t10)	0.89[a]	1.88[b]	1.74[b]	0.06
C18:1 c9	13.05[a]	18.77[b]	23.28[c]	0.34
Other C18:1 c[3]	0.64[a]	1.29[c]	1.08[b]	0.03
C18:2 c9c12	1.57[a]	1.64[a]	1.30[b]	0.02
C18:2 c9t11	0.61[a]	0.94[c]	0.83[b]	0.02
C18:3 c9c12c15	0.75[a]	1.96[b]	0.66[a]	0.04
C20:0	0.17[a]	0.19[a]	0.26[b]	0.01
C10:0+C12:0+C14:0	21.23[a]	16.53[b]	17.73[b]	0.320
Σ (C10-C17)[4]	48.68[a]	37.91[b]	40.28[b]	0.667
Σ C18[5]	24.03[a]	40.45[b]	45.17[c]	0.691
C14:1 c9/C14:0	0.020[a]	0.014[b]	0.015[b]	0.0003
C16:1 c9/C16:0	0.028[a]	0.022[b]	0.023[b]	0.0003
C18:1 c9/C18:0	2.43[a]	1.66[c]	1.81[b]	0.026
C18:2 c9c12/C18:3 c9c12c15	2.13[a]	0.86[b]	2.28[a]	0.046

[a,b,c]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Standard error of least square means (n = 42)

[2]Other C18:1t = t4, t5, t6, t7, t8, and t9.

[3]Other C18:1c = c11, c12, c13, c14 (including the t16), and c15 (including the t17).

[4]Sum includes all the C10 to C17 fatty acids, including those not presented in the table.

[5]Sum includes all the C18 fatty acids, including those not presented in the table.

Table 7. Activities of lipoprotein lipase, acetyl-CoA carboxylase, fatty acid synthase, glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme, stearoyl-CoA desaturase, and glycerol-3-phosphate dehydrogenase (G3PDH) in the mammary gland of lactating goats fed a hay-based diet supplemented or not (control) with 3.6% lipid of formaldehyde-treated linseed (FLS) or oleic sunflower oil (OSO).[1]

Item	Control (n = 4)	FLS (n = 5)	OSO (n = 5)
Lipoprotein lipase	45 ± 9	34 ± 7	51 ± 6
Acetyl-CoA carboxylase	7.54 ± 1.68	5.75 ± 1.69	5.05 ± 1.07
Fatty acid synthase	87 ± 26	60 ± 12	87 ± 13
G6PDH	165 ± 29	201 ± 23	204 ± 9
Malic enzyme	2.67 ± 1.42	2.37 ± 0.44	3.43 ± 1.39
Stearoyl-CoA desaturase	0.198 ± 0.009[a]	0.129 ± 0.018[ab]	0.155 ± 0.011[b]
G3PDH	137 ± 32	129 ± 20	136 ± 6

[a,b,c]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Values represent the mean ± SE of 4, 5, or 5 observations for Control, FLS, and OSO, respectively; activities expressed as nmol/min per mg of protein.

the endogenous synthesis of oleic acid from stearic acid within the mammary gland. This result is in accordance with the level of C18:3 (n-3) intake which was similar for control and OSO (4.14 and 4.03 g/d, respectively) and about 11-fold higher for FLS (46.13 g/d). The OSO diet led to a significant ($P < 0.05$) increase of *trans*-11 (+ *trans*-10) C18:1 and *cis*-9, *trans*-11 C18:2 percentages in milk fat compared with control; FLS was significantly ($P < 0.05$) higher than OSO for these 2 fatty acids. When expressed in terms of secretion of *cis*-9, *trans*-11 C18:2, diets ranked: control < OSO < FLS, whereas for *trans*-11 (+ *trans*-10) C18:1 OSO and FLS led to a similar secretion, which was higher than in control.

The FLS and OSO diets resulted in lower *cis*-9 C16:1/C16:0, *cis*-9 C14:1/C14:0 ratios compared with the control diet ($P < 0.05$). The *cis*-9 C18:1/18:0 ratio was significantly lower with FLS than with OSO, which was lower than the control ($P < 0.05$). The FLS diet led to a lower C18:2 (n-6)/C18:3 (n-3) than OSO and control (Table 6).

Mammary Metabolism

Stearoyl-CoA desaturase activity decreased with both FLS and OSO, but significance was reached only with OSO compared with control (Table 7). Lipoprotein lipase activity tended ($P = 0.09$) to decrease with FLS compared with OSO, which was close to control. The diet had no effect on G6PDH, ME, ACC, FAS, and G3PDH activities (Table 7).

Stearoyl-CoA desaturase mRNA in the mammary gland was significantly ($P < 0.05$) lower with FLS compared with control, and tended (P = 0.07) to be lower with OSO compared with control (Table 8). Lipoprotein lipase mRNA level increased significantly ($P < 0.05$) with OSO compared with control.

DISCUSSION

DMI and Milk Production and Composition

Our results on milk fat and protein contents, and milk yield (Table 2) are consistent with data from the literature indicating that, conversely to what was observed in dairy cows (Chilliard, 1993), a fat-supplemented diet to goats sharply increased the percentage of milk fat, had a variable effect on protein content, and did not increase milk yield (Chilliard et al., 2003a). The increase in milk fat content and yield with OSO and FLS is in accordance with the net increase in the fatty acids brought to the mammary gland due to the lipid supplement in the diet. This increase in milk fat content of +6.6 and +7.4 g/kg, respectively, for FLS and OSO, was comparable with the +9.2 g/kg obtained by Mir et al. (1999) when goats were fed 4% canola oil, and the +4.5 g/kg obtained by Daccord (1987) when goats were fed 20% extruded soybean. Moreover, these results are in accordance with several other results reported in the review of Chilliard et al. (2003a).

Plasma Metabolites

The lack of significant variation of serum insulin and glucose levels between control and supplemented diets (Table 3) is in accordance with the absence of consistent effect of fat-supplemented diets on these parameters in dairy cows (review by Chilliard, 1993) and in goats (Chilliard et al., 2004). We did not detect any variation in plasma NEFA levels between diets (Table 3). This result is in accordance with Morand-Fehr et al. (1987) showing that plasma NEFA in lactating goats was not influenced by diet differing in type and level of fat, contrasting with the increase of plasma NEFA observed in cows receiving dietary fat (review by Chilliard, 1993).

Table 8. Levels of mRNA of lipoprotein lipase, fatty acid synthase, acetyl-CoA carboxylase, and stearoyl-CoA desaturase genes in the mammary gland of lactating goats fed a hay-based diet supplemented or not (control) with 3.6% lipid of formaldehyde-treated linseed (FLS) or oleic sunflower oil (OSO).[1]

Item	Control (n = 4)	FLS (n = 5)	OSO (n = 5)
Lipoprotein lipase	116 ± 12[b]	146 ± 32[ab]	175 ± 15[a]
Acetyl-CoA carboxylase	2.67 ± 0.21	2.13 ± 0.57	2.36 ± 0.37
Fatty acid synthase	1.67 ± 0.32	1.65 ± 0.59	1.32 ± 0.14
Stearoyl-CoA desaturase	20.2 ± 3.9[a]	9.21 ± 0.55[b]	11.6 ± 1.99[ab]

[a,b,c]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Values represent the mean ± SE of 4, 5 or 5 observations for Control, FLS and OSO, respectively; level of mRNA is expressed relative to cyclophylin gene expression level.

Adipose Tissue Lipid Metabolism

Lipoprotein lipase, FAS, G6PDH, and G3PDH activities in perirenal adipose tissue were not affected by the dietary treatments, although G3PDH activity tended to increase with FLS or OSO compared with control. Conversely, ME activity increased with FLS and tended to increase with OSO. Thus, we did not observe marked changes in adipose lipogenic enzymes, probably because the energy balance of our goats was positive and similar for the various diets, although in midlactation cows in positive energy balance, a duodenal infusion of a high dose (1.1 kg/d) of rapeseed oil decreased adipose tissue activities linked to FA synthesis and increased LPL activity (Chilliard et al., 1991). The results of the present study suggest that adipose tissue lipogenic or lipolytic (as reflected by plasma NEFA) pathways do not play an important role in the response of mammary lipid secretion to dietary lipid supplements.

Milk FA Composition and Yield

Although lipid supplementation to lactating goats' diets typically enhances milk fat percentage and yield, distinguishing goats from cows, the response in terms of milk FA composition is close to that observed in cows. The effect of vegetable lipid supplementation on goat milk FA composition depends on the nature of the lipid supplement (oil or seed and its FA composition) and its prior technological treatment (protected or not), its dose within the diet, and the type of forage forming the basal diet (Chilliard et al., 2003a). The milk fat percentage and secretion of C18:3 (n-3) were significantly higher with FLS compared with either control or OSO, in accordance with published studies reporting that feeding protected lipids to goats increased milk FA proportionally to their percentage in the lipid supplements. This result corroborates observations from our laboratory (Chilliard et al., 2003a) in which FLS (compared with crude linseed) allowed a 1.6-fold higher increase in the secretion of C18:3 (n-3) in milk, thus demonstrating the efficiency of the formaldehyde treatment. However, the apparent transfer rate (Figure 1) was low for C18:3 (n-3) from the FLS diet to milk (4.2%) compared with the transfer rate of total C18 FA (48.1%). This suggests that either the ruminal biohydrogenation of C18:3 (n-3) was high and gave rise to C18:1 and C18:2 isomers as well as C18:0, or that this FA is highly incorporated into plasma phospholipids and cholesterol ester, which are not readily taken up by the mammary gland. Furthermore, the high increase in C18:0 and *cis*-9 C18:1 milk secretion in FLS compared with control suggests a partial protection of linseed *cis*-9 C18:1 from ruminal biohydrogenation.

The apparent transfer rate of C18:2 (n-6) from the diet to milk (8.3%) was similar for FLS and OSO (Table 1 and Figure 1), as were the daily intake and secretion of this FA. For total C18, the apparent transfer rate from the diet to milk was 117, 48, and 55%, respectively, for the control, FLS, and OSO diets, for which the respective intake levels were 21, 84, and 82 g/d. This suggests that the highest is the intake of long-chain FA (total C18 FA), the lowest is the apparent transfer rate into milk, as observed in dairy cows for polyunsaturated fatty acids (**PUFA**) infused into the duodenum (Chilliard et al., 2000). This is probably due to higher utilization of these FA by nonmammary tissues, together with a high level of incorporation into phospholipid and cholesterol ester. Moreover, for the control diet, the transfer rate of total C18 FA was greater than 100%, suggesting that part of these FA secreted into milk comes from endogenous synthesis and release by peripheral tissues, particularly adipose tissues (Chilliard, 1993).

The highest percentage and secretion of *trans*-11 (+ *trans*-10) C18:1 and *cis*-9, *trans*-11 C18:2 in milk from FLS diet suggests a partial biohydrogenation of the dietary C18:3 (n-3) and C18:2 (n-6) (Harfoot and Hazlewood, 1988). However, FLS (like untreated linseeds) led to lower percentages of *trans*-C18:1 isomers and *cis*-9, *trans*-11 C18:2 in milk than that observed with free linseed oil (Chilliard et al., 2003a). On the other hand,

the highest percentage of C18:0 and *cis*-9 C18:1 (which represent respectively 14 and 25% of the milk fat) observed for OSO, could be explained by partial biohydrogenation of the dietary *cis*-9 C18:1 to C18:0 followed by its desaturation by the mammary SCD, as well as a partial preservation of *cis*-9 C18:1 from the rumen. Furthermore, the increase in milk long-chain FA secretion observed with FLS and OSO was accompanied by a significant decrease of medium-chain (C10 to C17) FA secretion (Table 6). These results are in accordance with other goat studies (see review by Chilliard et al., 2003a), where the decrease of mainly C8 to C16 in response to feeding lipid supplements could be attributed to the ruminal synthesis of potent inhibitors of the mammary de novo FA synthesis such as *trans*-C18:1 or *trans*-C18:2 isomers as well as to the dietary PUFA themselves, as discussed in the mammary metabolism section (see below). Moreover, secretion of C15:0 and C17:0 in milk, resulting from microbial FA metabolism in the rumen, decreased with FLS and OSO compared with control, which suggests that the addition of lipid supplements alters rumen metabolism.

Mammary Metabolism

The previous characterization of the caprine SCD mRNA (Bernard et al., 2001) and the development of molecular tools for studying SCD gene expression (Bernard et al., 2002), allowed us to study its mammary mRNA in the present study (Table 8). Moreover, the mammary SCD activities measured here are in accordance with previous data obtained in lactating cows (McDonald and Kinsella, 1973), sheep (Wahle, 1974), and goats (Bickerstaffe and Annison, 1970). Thus, the mRNA levels could be compared with the measure of mammary SCD activity itself or with the milk *cis*-9 C14:1/C14:0, *cis*-9 C16:1/C16:0, and *cis*-9 C18:1/C18:0 ratios (Table 6) representing a proxy for mammary desaturase activity. Globally, these parameters (SCD mRNA, SCD activity, and Δ^9-desaturation ratios) were highest for the control diet, and the 2 lipid supplemented diets decreased or tended to decrease them. Stearoyl-CoA desaturase mRNA followed a similar variation for milk *cis*-9 C14:1/C14:0, *cis*-9 C16:1/C16:0, and *cis*-9 C18:1/C18:0 for control and FLS diets. These results suggest that the various tools (mRNA, in vitro activity, and milk proxy ratios for SCD activity) used for studying the in vivo SCD regulation are positively, albeit not strongly, related. The differences observed between these parameters may be due to (1) the fact that the milk ratios of *cis*9 monounsaturated/saturated FA could be influenced by factors other than SCD activity, such as accuracy in the quantification of *cis*-9 isomers, a differential uptake of the different FA of the ratios by the mammary gland as well as their differential turnover and use by the mammary tissue itself, and (2) to the limits of SCD mRNA and in vitro activity to estimate in vivo SCD activity.

Lipid supplementation led to a decrease in both mammary SCD mRNA level and enzyme activity suggesting a negative regulation by dietary PUFA and long-chain FA or, for the unsaturated FA, by their ruminal biohydrogenation products. Hence, the effect of FLS on SCD mRNA could be partly attributed to dietary C18:3 (n-3) being protected from the rumen (Tables 5 and 6) or to *trans*-isomers of C18:1 and C18:2 (Chilliard et al., 2003b) produced in the rumen. Similarly, the observed effect of OSO on SCD activity might be partially attributed to *cis*-9 C18:1, as well as to its ruminal biohydrogenation products, as oleic acid could be isomerized in several *trans*-C18:1 isomers: *trans*-6, *trans*-7, and all *trans* in positions 9 to 16 including *trans*-10, as observed in microbial cultures from bovine rumen (Mosley et al., 2002), and in agreement with the increase in *trans*-11 (+ *trans*-10) C18:1 observed here with OSO (Tables 5 and 6). Then, these results (SCD mRNA and activity, and milk proxy ratios for SCD activity), suggest that isomerization and hydrogenation products of either *cis*-9 C18:1 or C18:3 (n-3) could also affect mammary SCD regulation.

Additionally, a negative effect of linolenic acid on goat mammary SCD activity has been observed in vitro (Bickerstaffe and Annison, 1970). The same can be said for fish oil (rich in long-chain n-3 FA) on SCD gene expression in the bovine mammary gland (Ahnadi et al., 2002). Thus, we may hypothesize a joint negative effect of (n-3) PUFA and *trans*-FA on SCD gene expression with FLS.

In the present experiment, the level of ACC and FAS activities observed in the goat mammary gland are in accordance with those observed previously in goats (Chilliard et al., 1986) and in cows (Piperova et al., 2000), and were not significantly affected by the lipid supplement as observed for the activities of enzymes associated with NADPH generation, G6PDH and ME, or glycerol-3-phosphate synthesis. Like their activities, FAS and ACC mRNA were not modified significantly by lipid supplement to the diet, although a decrease of 23 or 18% in the secretion of the sum of C10-C17 FA was observed, respectively, for FLS and OSO compared with control (Table 6). Nevertheless, although the mammary parameters have been measured during the last period of the experiment and present relatively high variations, the mRNA level and in vitro enzyme activities tended to decrease in the same proportion as the in vivo activity of the mammary gland to synthesize de novo FA.

The absence of a significant effect of FLS and OSO on ACC gene expression observed in the present study contrasts with rodents, in which a negative effect of diets rich in (n-3) and (n-6) PUFA was observed on the expression of lipogenic genes including ACC in liver and adipose tissue (Raclot and Oudart, 1999). This could be explained in part by the high biohydrogenation of PUFA. However, the high occurrence of *trans*-fatty acids in goat milk (Table 5 and Chilliard et al., 2003b, Ferlay et al., 2003) could have been expected to inhibit ACC and FAS. Indeed, in bovine mammary cell cultures, Jayan and Herbein (2000) showed that *trans*-11 C18:1 reduced activities of ACC and FAS compared with oleic acid, and recently in the mammary gland of rats fed a diet containing a mixture of *trans*-isomers, an impairment of lipid biosynthesis was observed (Assumpcao et al., 2002). In cows, Piperova et al. (2000), using a milk fat-depressing diet (25:70% forage/concentrate, supplemented with 5% soybean oil), observed a reduction in ACC mRNA abundance and activity, and in FAS activity in mammary tissue, associated with a dramatic decrease of 59% in C10-C16 FA secretion (g/d). Similarly, in cows, Ahnadi et al. (2002), using diets supplemented with fish oil, observed a decrease in ACC and FAS mRNA level in the mammary gland together with a decrease of 38% in C4-C16 FA secretion. However, in the present study, the slight decrease (~20%) in milk C4-C16 FA secretion with lipid supplemented diets partially agreed with the slight variation of ACC and FAS mRNA and activities as well as ME and G6PDH activities.

Part of the differences observed in the literature concerning the response of ACC and FAS to PUFA/lipid supplementation between cows (Piperova et al., 2000; Ahnadi et al., 2002) and goats (the present study) may be explained by species differences in the response to lipid supplement (see above), as well as by the nature and composition of the FA (fish oil vs. vegetable oil) and the form (seeds vs. free oil) of the lipid supplement. These differences between cows and goats suggest specific differences of FA ruminal metabolism or mammary metabolism between ruminant species.

Mammary LPL mRNA was increased with OSO compared with control, and LPL activity tended to increase for OSO compared with FLS. Conversely, milk LPL activity sharply decreased with FLS and more markedly with OSO (Table 3). This decrease in milk LPL activity when lipid supplements were added to the diet has been previously observed (Chilliard et al., 2003a). We hypothesize that milk LPL decreased when supplemental lipids were fed because more mammary LPL enzyme was directed toward the capillary lumen to allow the uptake of blood triglyceride arising from digestive absorption, and less enzyme was transported within the mammary alveolar cells toward the milk, in accordance with the models for LPL transport in the mammary gland proposed by Jensen et al. (1994) and Chilliard et al. (2003a).

Lipoprotein lipase activity in rat mammary gland is enhanced by a high dietary lipid intake during lactation (Del Prado et al., 1999), whereas LPL mRNA in cow mammary gland was not modified by addition of 1.5 or 3% of protected fish oil to the diet DM (Ahnadi et al., 2002). In the present experiment, the increase of 65 and 83% in the secretion of long-chain FA (C18) observed respectively for FLS and OSO, compared with control, together with the absence of effect of the lipid supplemented diets on LPL activity measured over the last period of the experiment, suggest that the limiting factor in the uptake of long chain FA by the mammary gland is not always linked to the in vitro LPL activity. Thus, the availability of C18 FA in plasma very low density lipoproteins and chylomicrons could play an important role (Gagliostro et al., 1991), as well as the partitioning of LPL between basal membrane or intracellular space within the secretory cells (as discussed above).

CONCLUSIONS

Our results demonstrate that the mechanisms by which lipid supplementation modulates FA secretion are related to the level of mammary expression of genes encoding enzymes involved in FA uptake and de novo FA synthesis in goats. Probably, other factors such as the cellular localization of the enzymes and the availability of the substrates (such as long-chain FA in the very low density lipoproteins for LPL) are limiting in addition to the in vitro activity of the enzymes that control these pathways. Milk ratios representing a proxy for SCD activity decreased with lipid supplementation and were related to SCD mRNA and activity.

Feeding formaldehyde-treated linseed or oleic sunflower oil to lactating goats changed milk fat yield and FA composition with a slight decrease of the secretion of medium-chain FA (C10-C17) and a dramatic increase in the secretion of C18 FA. The decrease in saturated FA and the increase in *cis*-9, *trans*-11 C18:2 observed with FLS and OSO and, for FLS the lowest C18:2 (n-6)/C18:3 (n-3) ratio, could contribute to improve the nutritional value of goat milk.

ACKNOWLEDGMENTS

We thank A. Ollier, A. Combeau, and their team for the management of the animals, R. Jailler and his team for slaughtering the animals, and INZO company (Chateau-Thierry, France) for supplying the formaldehyde-

treated linseed. We thank P. Capitan, J. Fléchet, M. Tourret, D. Bany, E. Guillin, and V. Cromarias for their technical assistance. We thank M. Bonnet for giving the lipoprotein lipase and cyclophylin probes and primers for mRNA quantification, J. Mourot and P. Peiniau for their helpful advice in the ACC assay, and D. Catheline for his training in the SCD assay.

REFERENCES

Ahnadi, C. E., N. Beswick, L. Delbecchi, J. J. Kennelly, and P. Lacasse. 2002. Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes. J. Dairy Res. 69:521–531.

AOAC. 1997. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

Assumpcao, R. P., F. D. Santos, C. L. Setta, G. F. Barreto, I. E. Matta, D. Estadella, V. B. Azeredo, and M. G. Tavares do Carmo. 2002. *Trans*-fatty acids in maternal diet may impair lipid biosynthesis in mammary gland of lactating rats. Ann. Nutr. Metab. 46:169–175.

Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. Anal. Biochem. 70:241–250.

Bernard, L., C. Leroux, Y. Chilliard, and P. Martin. 2002. Characterisation of the caprine Stearoyl-CoA Desaturase gene and its mammary transcript. Development of a method of mRNA quantification by real time RT-PCR. Reprod. Nutr. Dev. 42:499–500.

Bernard, L., C. Leroux, H. Hayes, M. Gautier, Y. Chilliard, and P. Martin. 2001. Characterisation of the caprine stearoyl-CoA desaturase gene and its mRNA showing an unusually long 3′-UTR sequence arising from a single exon. Gene 281:53–61.

Bickerstaffe, R., and E. F. Annison. 1970. The desaturase activity of goat and sow mammary tissue. Comp. Biochem. Physiol. 35:653–665.

Bonnet, M., C. Leroux, Y. Faulconnier, J. F. Hocquette, F. Bocquier, P. Martin, and Y. Chilliard. 2000. Lipoprotein lipase activity and mRNA are up-regulated by refeeding in adipose tissue and cardiac muscle of sheep. J. Nutr. 130:749–756.

Cathala, G., J. F. Savouret, B. Mendez, B. L. West, M. Karin, J. A. Martial, and J. D. Baxter. 1983. A method for isolation of intact, translationally active ribonucleic acid. DNA 2:329–335.

Chakrabarty, K., and G. A. Leveille. 1969. Acetyl-CoA carboxylase and fatty acid synthase activities in liver and adipose tissue of meal-fed rats. Proc. Soc. Exp. Biol. Med. 131:1051–1054.

Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: A review. J. Dairy Sci. 76:3897–3931.

Chilliard, Y., L. Bernard, J. Rouel, A. Ferlay, P. Gaborit, K. Raynal-Ljutovac, and A. Lauret. 2004. Effet de l'alimentation et de sa composition en matière grasse sur la qualité du lait et des produits laitiers caprins (aptitude technologique, qualité sensorielle) et la production d'acides gras présentant un avantage pour la santé du consommateur. INRA, Theix, France.

Chilliard, Y., C. Delouis, M. C. Smith, D. Sauvant, and P. Morand-Fehr. 1986. Mammary metabolism in the goat during normal or hormonally induced lactation. Reprod. Nutr. Dev. 26:607–615.

Chilliard, Y., A. Ferlay, R. M. Mansbridge, and M. Doreau. 2000. Ruminant milk fat plasticity: Nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Ann. Zootech. 49:181–205.

Chilliard, Y., A. Ferlay, J. Rouel, and G. Lamberet. 2003a. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci. 86:1751–1770.

Chilliard, Y., G. Gagliostro, J. Fléchet, R. Lefaivre, and I. Sebastian. 1991. Duodenal rapeseed oil infusion in early and midlactation cows. 5. Milk fatty acid and adipose tissue lipogenic activities. J. Dairy Sci. 74:1844–1854.

Chilliard, Y., J. Rouel, J. M. Chabosseau, P. Capitan, P. Gaborit, and A. Ferlay. 2003b. Interactions between raygrass preservation and linseed oil supplementation and composition, including trans and conjugated fatty acids. Page 343 in Proc 54th Annu. Mtg. European Association for Animal Production, Rome, Italy. Y. van der Honing, ed. Wageningen Acad. Publ., Wageningen, the Netherlands.

Corl, B. A., L. H. Baumgard, D. A. Dwyer, J. M. Griinari, B. S. Philipps, and D. E. Bauman. 2001. The role of delta(9)-desaturase in the production of *cis*-9, *trans*-11 CLA. J. Nutr. Biochem. 12:622–630.

Daccord, R. 1987. Effect of addition of animal or vegetable fat to a hay based diet on digestibility and nitrogen balance in the lactating goat. Ann. Zootech. 36:329. (Abstr.)

Del Prado, M., S. Villalpando, J. Gordillo, and H. Hernandez-Montes. 1999. A high dietary lipid intake during pregnancy and lactation enhances mammary gland lipid uptake and lipoprotein lipase activity in rats. J. Nutr. 129:1574–1578.

Faulconnier, Y., M. Thévenet, J. Fléchet, and Y. Chilliard. 1994. Lipoprotein lipase and metabolic activities in incubated bovine adipose tissue explants: Effects of insulin, dexamethasone, and fetal bovine serum. J. Anim. Sci. 72:184–191.

Ferlay, A., J. Chabrot, Y. Elmeddah, and M. Doreau. 1993. Ruminal lipid balance and intestinal digestion by dairy cows fed calcium salts or rapeseed oil fatty acids or rapeseed oil. J. Anim. Sci. 71:2237–2245.

Ferlay, A., and Y. Chilliard. 1999. Effects of the infusion of non-selective beta-, and selective beta1- or beta2-adrenergic agonists, on body fat mobilisation in underfed or overfed non-pregnant heifers. Reprod. Nutr. Dev. 39:409–421.

Ferlay, A., J. Rouel, J. M. Chabosseau, P. Capitan, K. Raynal-Ljutovac, and Y. Chilliard. 2003. Interactions between raygrass preservation and high-oleic sunflower oil supplementation on goat milk composition, including trans and conjugated fatty acids. Page 343 in Proc 54th Annu. Mtg. European Association for Animal Production, Rome, Italy. Y. van der Honing, ed. Wageningen Acad. Publ., Wageningen, the Netherlands.

Folch, J., M. Lees, and G. H. S. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Gagliostro, G., Y. Chilliard, and M. J. Davicco. 1991. Duodenal rapeseed oil infusion in early and midlactation cows. 3. Plasma hormones and mammary apparent uptake of metabolites. J. Dairy Sci. 74:1893–1903.

Glass, R. L. 1971. Alcoholysis, saponification and the preparation of fatty acid methyl esters. Lipids 6:919–925.

Grosclaude, F., G. Ricordeau, P. Martin, F. Remeuf, L. Vassal, and J. Bouillon. 1994. Du gène au fromage: Le polymorphisme de la caséine α-s1 caprine, ses effets, son évolution. INRA Prod. Anim. 7:3–19.

Harfoot, C. G., and G. P. Hazlewood. 1988. Lipid metabolism in the rumen. Pages 285–322 in The rumen microbial ecosystem. P. N. Hobson, ed. Elsevier Science Publishers, London, UK.

Jayan, G. C., and J. H. Herbein. 2000. Healthier dietary fat using *trans*-vaccenic acid. Nutr. Food Sci. 30:304–309.

Jensen, D. R., S. Gavigan, V. Sawicki, D. L. Witsell, R. H. Eckel, and M. C. Neville. 1994. Regulation of lipoprotein lipase activity and mRNA in the mammary gland of the lactating mouse. Biochem. J. 298:321–327.

Legrand, P., D. Catheline, M. C. Fichot, and P. Lemarchal. 1997. Inhibiting delta9-desaturase activity impairs triacylglycerol secretion in cultured chicken hepatocytes. J. Nutr. 127:249–256.

Massaro, M., M. A. Carluccio, and R. De Caterina. 1999. Direct vascular antiatherogenic effects of oleic acid: A clue to the cardioprotective effects of the Mediterranean diet. Cardiologia 44:507–513.

McDonald, T. M., and J. E. Kinsella. 1973. Stearyl-CoA desaturase of bovine mammary microsomes. Arch. Biochem. Biophys. 156:223–231.

Mir, Z., L. A. Goonewardene, E. Okine, S. Jaegar, and H. D. Scheer. 1999. Effect of feeding canola oil on constituents, conjugated linoleic acid (CLA) and long chain fatty acids in goats milk. Small Rumin. Res. 33:137–143.

Morand-Fehr, P., P. Bas, and D. Sauvant. 1987. Influence de la nature et de la quantité de lipides ajoutés à la ration sur la sécrétion de

lait et de la matière grasse chez la chèvre. Reprod. Nutr. Dev. 27:309–310.

Mosley, E. E., G. L. Powell, M. B. Riley, and T. C. Jenkins. 2002. Microbial biohydrogenation of oleic acid to trans isomers in vitro. J. Lipid Res. 43:290–296.

Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753–1771.

Piperova, L. S., B. B. Teter, I. Bruckental, J. Sampugna, S. E. Mills, M. P. Yurawecz, J. Fritsche, K. Ku, and R. A. Erdman. 2000. Mammary lipogenic enzyme activity, trans fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130:2568–2574.

Raclot, T., and H. Oudart. 1999. Selectivity of fatty acids on lipid metabolism and gene expression. Proc. Nutr. Soc. 58:633–646.

SAS Institute. 2000. SAS User's guide: Statistics, Version 8 ed. SAS Inst., Inc., Cary, NC.

Wahle, K. W. 1974. Desaturation of long-chain fatty acids by tissue preparations of the sheep, rat and chicken. Comp. Biochem. Physiol. B 48:87–105.