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Comparison of mammary lipid metabolism in dairy cows and goats fed diets supplemented with starch, plant oil, or fish oil

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ABSTRACT

A direct comparison of cow and goat performance and milk fatty acid (FA) responses to diets known to induce milk fat depression in the bovine has suggested interspecies differences in rumen and mammary lipid metabolism. Thus, this study was conducted to infer some potential mechanisms responsible for the differences in mammary lipogenesis due to diet and ruminant species. To meet this objective, 12 cows and 15 goats were fed a basal diet (control), a similar diet supplemented with 2.2% fish oil (FO), or a diet containing 5.3% sunflower oil and additional starch (+38%; SOS) according to a 3 × 3 Latin square design with 26-d experimental periods. Milk yield, milk composition, FA profile, and FA secretion were measured. On the last day of each period, the mRNA abundance of 19 key genes in mammary metabolism or the enzyme activity or both were measured in mammary tissue sampled by biopsy or at slaughter or both. The results show significant differences in the response of cows and goats to the dietary treatments. In cows, milk fat content and yield were lowered by FO and SOS (−31%), whereas only FO decreased milk fat content in goats (−21%) compared with the control. In cows and to a lesser extent in goats, FO and SOS decreased the secretion of <C16 and C16 FA, and FO lowered >C16 FA output (mmol/kg of BW). However, SOS increased the secretion of >C16 FA in goats. These changes in milk fat content and FA secretion were not associated with modifications in mammary expression or the activity of 19 proteins involved in the major lipogenic pathways. This absence of variation may be attributable to posttranscriptional regulation for these genes or related to the time of sampling of the mammary tissue relative to the previous meal and milking. Otherwise, the abundances of 15 mRNA among the 19 encoding for genes involved in lipid metabolism in the mammary gland were different among species, with 9 more abundant in cows (*FASN, FADS1, SCD1, GPD1, LALBA, SREBF1, LXRA, PPARA,* and *PPARG1*) and 6 more abundant in goats (*G6PD, GPAM, SCD5, XDH, CSN2,* and *SP1*). Similarly, a significant effect of the species was observed in the 4 enzyme activities measured; glycerol-3-phosphate dehydrogenase and malic enzyme were higher in cows, and FA synthase and glucose-6-phosphate dehydrogenase activities were higher in goats. In conclusion, the differences between cow and goat performance and milk FA responses to the FO and SOS treatments were not related to changes in the measured mammary lipogenic gene expression. Furthermore, the data provide evidence that the major mammary lipogenic pathways differ between the caprine and the bovine, whose biological significance remains to be unraveled.

Key words: cow, goat, lipogenic gene expression, milk fatty acid, milk fat depression

INTRODUCTION

In milk, fat is the major energy constituent and represents a significant proportion of the total energy requirements for lactation in ruminants. Moreover, fat is an important component contributing to the technological, organoleptic, and nutritional properties of milk (Palmquist et al., 1993; Chilliard and Ferlay, 2004). Nutrition is the major environmental factor that regulates milk fat secretion and composition and constitutes a rapid, reversible, and efficient means to modulate milk fat synthesis and composition (Chilliard et al., 2007). Thus, a better understanding of milk fat synthesis regulation within the mammary gland is central to the development of nutritional strategies to limit milk energy secretion, improve the energy balance of lactating ruminants, and enhance the nutritional value of milk for human consumers. Under certain dietary conditions, such as starch-rich diets or the addition of plant oil or marine oils, dairy ruminants may experience milk fat depression (**MFD**), and milk fat concentration and yield may decrease dramatically (up to 50%; Bauman and Griinari, 2003). Several theories have been

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proposed to explain the causes of MFD (Bauman and Griinari, 2003; Shingfield and Griinari, 2007), with the biohydrogenation (**BH**) theory being the most universal. This theory attributes diet-induced MFD to an inhibition of mammary lipogenesis by specific fatty acid (**FA**) intermediates that form in the rumen on certain diets due to alterations in ruminal BH pathways (Bauman and Griinari, 2003; Shingfield et al., 2010). The BH theory provides a basis for explaining most cases of MFD on starch-rich diets or with plant oil in cows via a direct inhibition of mammary lipogenesis by BH intermediates with confirmed or putative antilipogenic effects [*trans*-10,*cis*-12 CLA (Baumgard et al., 2000), *cis*-10,*trans*-12 CLA (Sæbø et al., 2005), and *trans*-9,*cis*-11 CLA (Perfield et al., 2007)]. However, these intermediates do not, in isolation, explain MFD in cows or sheep fed diets containing marine oils (Loor et al., 2005; Gama et al., 2008; Toral et al., 2010). Thus, for dietary conditions with marine oil, Shingfield and Griinari (2007) proposed also including the role of changes in the availability of preformed long-chain FA to the mammary gland in the explanation of MFD. This would involve both a shortage of 18:0 for endogenous *cis*-9 18:1 synthesis in the mammary gland and an increase in the supply of *trans* FA formed in the rumen, which would increase the milk fat melting point and thereby lower the rate of fat removal in mammary epithelial cells (Loor et al., 2005; Gama et al., 2008).

An indirect comparison of cow and goat performance and milk FA responses to diets known to induce MFD in the bovine reveals relevant species-by-diet interactions, with goats being less sensitive to diet-induced MFD (Shingfield et al., 2010; Toral et al., 2014) even though recent studies have demonstrated their susceptibility when fed marine lipids at a relatively high dose (2.2% DM; Toral et al., 2015). The reasons for the differential lipogenic responses between these ruminant species are not well understood, but based on indirect comparisons of milk FA composition, differences in ruminal BH and mammary lipid metabolism (Chilliard et al., 2007, 2014; Shingfield et al., 2010) could be implicated. Faced with the absence of direct interspecies comparisons of diet-induced MFD among these species, a comparative study with lactating cows and goats was undertaken to determine the mechanisms underlying the differences of mammary lipogenic responses between these ruminant species. From this study, animal performance responses including milk fat yield, FA composition (Toral et al., 2015), and rumen metabolism (Toral et al., 2016) were reported and revealed relevant interspecies differences and species-by-diet interactions. The present study aimed to provide further insight into the mechanisms regulating mammary lipogenesis in ruminants. In the mammary gland, milk fat is controlled via the expression of a network of lipogenic genes (Bionaz and Loor, 2008), and alterations in mammary lipogenic gene expression may partly explain the basis for diet-induced MFD. However, the underlying mechanisms might differ depending on factors such as dietary conditions or animal species.

On this basis, a direct comparative study with lactating cows and goats was undertaken to test the hypotheses that (1) the mammary mechanisms underlying MFD induced by marine lipid supplements or by diets containing high amounts of starch and plant oils are different and (2) mammary lipid metabolism responses vary between animal species. To meet this objective, cows and goats were fed a basal diet (control), a similar diet supplemented with fish oil (**FO**), or a diet containing sunflower oil and additional starch from wheat (**SOS**). Then, changes in animal performance and milk FA, the mammary expression of several genes involved in the major lipogenic pathways, and the activity of a few enzymes related to corresponding proteins were measured and used to infer the potential mechanisms responsible for differences in the regulation of mammary lipogenesis due to diet and ruminant species.

MATERIALS AND METHODS

Animals, Experimental Design, Diets, and Management

All procedures involving animals were approved by the Animal Care Committee of INRA in accordance with the guidelines established by European Union Directive 2010/63/EU. The details of the experimental design have been described in Toral et al. (2015). Briefly, 12 multiparous nonpregnant Holstein cows and 15 multiparous nonpregnant Alpine goats were allocated to 1 of 3 groups (4 cows and 5 goats each) and used in a replicated 3 × 3 Latin square to test the effects of 3 treatments during three 25-d experimental periods. One goat had to be withdrawn from the experiment because it suffered from diarrhea. All animals were offered grass hay ad libitum supplemented with concentrates (control, FO, or SOS). Formulation of experimental concentrates and chemical composition of concentrates and grassland hay were described in Toral et al. (2015). Briefly, the control concentrate was based on (% DM) cracked corn grain (54.9), pelleted dehydrated alfalfa (29.4), soybean meal (14.3) and a mineral–vitamin premix (1.4). In the FO and SOS concentrates, both FO (3.6% DM) and sunflower oil (9.0% DM) replaced alfalfa pellets on a proportionate basis and were mixed manually with other ingredients immediately before being fed out. The FO represented 2.2% of total DMI, and the sunflower oil represented 5.3% of total DMI.

The remaining alfalfa pellets and part of the corn grain were replaced by flattened wheat grain (37.4% DM) in the SOS concentrate. Hay refusals were weighed daily and used to adjust the amounts of concentrate offered the following day to maintain the targeted dietary forage to concentrate ratio (40:60 on a DM basis). Diets were offered as 2 equal meals at 0830 and 1600 h. The formulation, chemical composition, and FA profile of the concentrates and hay have been reported previously (Toral et al., 2015). The experimental diets were formulated to be isoproteic (139 g of CP/kg of DM). The mean starch concentrations were 232, 239, and 325 g/kg of DM and the mean NDF concentrations were 365, 349, and 296 g/kg of DM for the control, FO, and SOS diets, respectively. Fish oil and sunflower oil were supplied at 400 and 953 g of FA/d, respectively, in cows and 48 and 114 g of FA/d, respectively, in goats. The animals had access to a constant supply of fresh water and were milked at 0800 and 1530 h.

Measurement and Sampling

Feed intake, the chemical composition of experimental diets, and milk yield were determined for each experimental period according to sampling protocols and analytical procedures outlined elsewhere (Toral et al., 2015). Samples of milk were collected individually from all animals over 4 consecutive milkings starting at 0800 h on d 23 of each experimental period for the measurement of fat, protein, and lactose with preservative (bronopol-B2; Trillaud, Surgères, France). Unpreserved samples of milk were also collected over 2 consecutive milkings starting at 0800 h on d 24 of each experimental period, stored at −20°C, composited according to milk yield, and submitted for the determination of FA composition (Toral et al., 2015).

On d 25 of each experimental period, mammary tissue was collected under sterile conditions using a biopsy instrument (AgResearch Ruakura, Ruakura Agricultural Center, Hamilton, New Zealand; previously described by Farr et al., 1996) for cows and the Bard Monopty instrument (12 g × 10 cm; Bard Biopsy Systems, Tempe, AZ; according to the manufacturer's instructions) for goats. Mammary biopsies were obtained before the morning milking (i.e., after an overnight without concentrate distribution but with ad libitum hay) and before morning feeding as previously described for cows (Bernard et al., 2015b) and goats (Bernard et al., 2012).

Approximately 600 and 20 mg of mammary tissue for cows and goats, respectively, was collected from a midpoint on a rear quarter, alternating between the 2 rear quarters of the udder for the 3 periods. The tissue biopsies were rinsed in a 0.9% sterile saline solution and inspected visually to verify the homogeneity of the secretory tissue sampling. The biopsies were rapidly snap frozen in liquid N_2 and kept at −80°C until RNA extraction and enzyme assays for cows and RNA extraction for goats. The collection of tissue biopsies resulted in minimal bleeding, and milk appeared normal after 1 to 3 subsequent milkings. During this period, extreme care was taken during manual milking to remove possible blood clots lodged in the glands. No IMI or loss of milk production was encountered following mammary tissue biopsies.

At the end of the experiment (third period), the goats were slaughtered after the morning milking. Immediately after death, a sample of mammary tissue was collected under sterile conditions, frozen in liquid N_2, and stored at −80°C for enzyme assays.

RNA Isolation and Real-Time Reverse Transcription PCR

Total RNA was prepared through the homogenization of approximately 80 to 100 mg of cow mammary tissue and 20 mg of goat mammary tissue in 1 and 0.35 mL of TRIzol reagent (Invitrogen, Carlsbad, CA), respectively, followed by isolation using the Pure Link RNA mini kit isolation system (Invitrogen) according to the instructions of the manufacturer. Potential contaminating genomic DNA was removed through a DNase treatment step (RNase-free DNase set no. 79254; Qiagen, Hilden, Germany). Concentrations of RNA were determined by measuring absorbance at 260, 280, and 320 nm using a NanoDrop spectrophotometer (ND-1000; Labtech, Palaiseau, France). Average RNA integrity was determined using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and was 7.8 (SD = 0.41) and 8.7 (SD = 0.32) for mammary RNA from cows and goats, respectively.

Using total RNA isolated from the mammary biopsy samples, reverse transcription was performed from 2 μg of purified total RNA using the high-capacity RNA-to-cDNA kit containing deoxynucleotide triphosphates, random octamers, and oligo dT-16 (Applied Biosystems, Foster City, CA) in a final volume of 20 μL. The samples were stored at −20°C.

The mRNA abundance of the following 19 candidate genes was measured via quantitative reverse transcriptase PCR: acetyl-CoA carboxylase-α (*ACACA*), FA synthase (*FASN*), and glucose-6-phosphate dehydrogenase (*G6PD*) involved in de novo FA synthesis; FA translocase (*CD36*), FA binding protein 3, muscle and heart (*FABP3*), and solute carrier family 2 (facilitated glucose transporter) member 1 (*SLC2A1*) involved in FA uptake for the 2 former and glucose uptake for the latter; stearoyl-CoA desaturase 1 (*SCD1*), stearoyl-CoA

desaturase 5 (*SCD5*), and FA desaturase 1 (*FADS1*) involved in FA desaturation; glycerol-3-phosphate dehydrogenase 1 (*GPD1*) and glycerol phosphate acyltransferase (*GPAM*) involved in triglyceride synthesis; α-LA (*LALBA*) and CN-β (*CSN2*), which are 2 major proteins in the milk; xanthine dehydrogenase/oxidase (*XDH*), which is one of the major proteins in the milk fat globule membrane; and the transcription factors sterol regulatory element binding transcription factor 1 (*SREBF1*), liver x receptor alpha (*LXRA*), peroxisome proliferator-activated receptor alpha (*PPARA*), peroxisome proliferator-activated receptor gamma (*PPARG1*), and Sp1 transcription factor (*SP1*), which are involved in the regulation of lipogenic gene expression. To account for variation in RNA integrity, RNA quantification, and cDNA synthesis, the mRNA abundance was normalized using the geometric mean of 3 housekeeping genes [ribosomal protein, large, P0 (*RPLP0*), ubiquitously expressed transcript (*UXT*), and eukaryotic translation initiation factor 3 subunit K (*EIF3K*)], which were identified as suitable internal controls for interspecies comparison among several tested (Bonnet et al., 2013). The mRNA abundance was quantified in duplicate via real-time quantitative reverse transcriptase PCR using the StepOnePlus real-time PCR system (Applied Biosystems), SYBR Green dye (TF Power SYBRGreen PCR Master Mix; Applied Biosystems), or a fluorescent TaqMan probe (TF TaqManFast Universal PCR Master Mix; Applied Biosystems) according to the manufacturer's instructions and with specific primers and probes (Supplemental Table S1; https://doi.org/10.3168/jds.2017-12789). Specific primers and probes were designed on a consensus cDNA fragment among species. Briefly, for SYBR Green technology, after an initial denaturing step (95°C for 15 min) the PCR mixture was subjected to the following 3-step cycle repeated 40 times: denaturing for 15 s at 94°C, annealing for 20 s at 58 or 60°C (depending of the primer pairs), and extension for 16 s at 72°C. Real-time PCR based on TaqMan probe technology was performed under the following conditions: 10 s at 95°C and 20 s at 58°C, which was repeated 40 times.

The PCR efficiency was 92.5% (SD = 1.63) for the 19 target genes and 92.1% (SD = 4.74) for the 3 reference genes. The abundance of candidate gene transcripts was expressed as the mRNA copy number relative to the geometric mean of the 3 housekeeping genes to account for variations in RNA integrity, RNA quantification, and cDNA synthesis and was log2 transformed.

Enzyme Assays

The activities of the following lipogenic enzymes were assayed as described by Bernard et al. (2005) in mammary gland samples: FA synthase (**FAS**; EC 2.3.1.85), malic enzyme (EC 1.1.1.40), and glucose-6-phosphate dehydrogenase (**G6PDH**; EC 1.1.1.49), which are involved in de novo lipogenesis, and glycerol-3-phosphate dehydrogenase (**G3PDH**; EC 1.1.1.8), which is involved in FA esterification.

Statistical Analysis

The mRNA abundance data were subjected to ANOVA for a 3 × 3 Latin square design (Kaps and Lamberson, 2009) using the MIXED procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC). The statistical model included the fixed effects of period, species, experimental diet, species × diet interaction, and order in which treatments were allocated to each animal and the random effect of animal nested within treatment order. For cows, enzyme activity data were subjected to ANOVA for a 3 × 3 Latin square design using the MIXED procedure in SAS. The statistical model included the fixed effects of period, experimental diet, and order in which treatments were allocated to each animal and the random effect of animal nested within treatment order. In goats, due to the availability of mammary tissue quantity, the enzyme activities data were determined exclusively in the last period and therefore were analyzed by 1-way ANOVA using the MIXED procedure in SAS (version 9.1). The statistical model included the fixed effect of experimental diet. The differences between means were evaluated using the "pdiff" option of the "LS means" statement in the MIXED procedure and adjusted for multiple comparisons using the Tukey-Kramer method. Pearson correlation coefficients were generated for associations between the mammary mRNA abundance among themselves and the concentration of specific FA in the milk using the CORR procedure in SAS. The treatment effects were declared significant at $P < 0.05$ and considered a trend toward significance at $P < 0.10$.

RESULTS

Diet Composition

The formulation of experimental concentrates and the chemical composition and FA profile of concentrate supplements and grassland hay are reported in Table 1. By design, grass hay was fed ad libitum, and the amount of concentrate offered was adjusted daily to maintain the target dietary forage to concentrate ratio (40:60 on a DM basis; for more details see Toral et al., 2015). The inclusion of oil resulted in a higher amount of ether extract in the FO and SOS treatments relative to the control (Table 1). Similarly, the starch content

(on a DM basis) was increased by approximately 40% in the SOS treatment compared with the control and FO treatments. By design, the inclusion of sunflower oil resulted in a greater intake of 18:0, *cis*-9 18:1, and 18:2n-6 in the SOS diet, with 18:2n-6 being the major FA, whereas the addition of FO increased the intake of 14:0, 16:0, *cis*-9 16:1, *cis*-11 18:1, 20:5n-3, 22:5n-3, and 22:6n-3 in the FO treatment (Table 2).

Animal Performance

The effects of the treatments on animal performance and milk composition are reported in Table 2, and more details are reported in Toral et al. (2015). The DMI per kilogram of BW was 11.8% higher ($P = 0.007$) in goats compared with cows, and the milk yield per kilogram of BW tended ($P = 0.052$) to be lower (−13%) for goats than for cows (Table 2). Milk fat and lactose contents were similar between species in the control, whereas milk protein content was higher ($P < 0.001$) for goats than for cows for all diets (average at 19.8%; Table 2). The daily milk yields of C16 (mmol/kg of BW) were lower in goats than in cows fed the control treatment ($P = 0.023$), but no differences ($P > 0.10$) were observed for the secretion of <C16 and >C16 FA (Table 2).

Compared with the control, the inclusion of oil supplements affected DMI expressed per kilogram of BW ($P < 0.001$) similarly in both species (Table 2). In goats, SOS increased ($P = 0.003$) milk lactose content compared with the control (Table 2). However, FO and SOS lowered ($P < 0.001$) milk fat content in cows (mean response at −31% compared with the control), whereas FO (but not SOS) decreased ($P < 0.001$) milk fat content in goats (mean response at −21% relative to the control; Table 2).

In cows and to a lesser extent in goats, FO and SOS tended to decrease the secretion (mmol/kg of BW) of <C16 FA ($P = 0.075$), and C16 FA output was reduced by SOS in both species and by FO in cows ($P < 0.05$). However, SOS increased secretion of >C16 FA in goats (Table 2).

Mammary Lipid Metabolism

Among the 19 mRNA encoding for genes involved in lipid metabolism in the mammary gland, species had a significant effect ($P < 0.05$) on the abundance of 15 of them, a tendency ($P < 0.10$) for significance for *ACACA* and *FABP3*, and no significant effect ($P > 0.10$) for *CD36* and *SLC2A1* (Table 3). On the 15 mRNA transcripts differentially expressed among species, 9 were more abundant in cows (*FASN, FADS1, SCD1, GPD1, LALBA, SREBF1, LXRA, PPARA, PPARG1*) and 6 were more abundant in goats (*G6PD, GPAM, SCD5, XDH, CSN2, SP1*; Table 3).

The dietary treatments had no effect ($P > 0.05$) on the abundance of the 19 mRNA encoding for genes involved in lipid metabolism in the mammary gland (Table 3), with the exception of a trend for *FADS1* ($P = 0.093$) and *SREBF1* ($P = 0.069$) mRNA abundances, which disappeared after multiple comparisons adjustment. Due to a species × diet interaction ($P < 0.05$), *PPARG1* mRNA was less abundant in the control and FO treatments in goats compared with SOS in goats and the 3 treatments in cows, but again, differences disappeared after multiple comparisons adjustment. Similarly, differences in *LXRA* did not reach the required level of significance after Tukey-Kramer adjustment, and *G6PD* was greater for the control and SOS in goats, lower for SOS in cows, and intermediate for the control and SOS in cows and for FO in goats.

In cows, the dietary treatments had no effect on enzyme activities in the mammary tissue collected at the end of each experimental period (n = 36) by biopsy (Table 4) except for a trend for a higher malic enzyme activity with FO compared with the control. In goats, the dietary treatments had no effect on enzyme activities in the mammary tissue collected at the end of the experiment (n = 14) at slaughter (Table 5). When comparing the data of cow (n = 12) and goat (n = 14) mammary tissues collected at the third experimental period, a significant effect of species was observed in the 4 enzyme activities measured in similar conditions. Indeed, in goats, FAS and G6PDH activities were approximately 10-fold and 3-fold higher compared with cows, respectively (Table 4). Conversely, G3PDH and malic enzyme were approximately 6-fold and 2-fold higher in cows compared with goats, respectively (Table 4).

DISCUSSION

To contribute to the development of feeding and management practices for altering milk FA composition and optimizing milk fat production, studies are undertaken to achieve a more complete understanding of nutritional regulation of milk fat secretion (Harvatine et al., 2009; Shingfield et al., 2010). The present study is the third part of a direct comparison experiment on dairy cows and goats examining performance and milk FA responses (Toral et al., 2015) and lipid metabolism at the level of the rumen (Toral et al., 2016) and the mammary gland (present study) to diets known to induce MFD in the bovine.

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

Item	Cows			Goats			SED[2]	*P*-value[3]		
	Control	FO	SOS	Control	FO	SOS		Sp	D	Sp × D
Ingredients, % of DM										
Grassland hay	42.4^{A}	40.9^{B}	40.4^{B}	41.8^{A}	39.5^{B}	39.5^{B}	0.11	0.108	0.005	0.860
Dehydrated alfalfa, pellets	17.0^{A}	15.1^{B}	—	17.1^{A}	15.7^{B}	—	0.02	0.120	<0.001	0.234
Corn grain, cracked	31.5^{B}	32.3^{AB}	22.6^{C}	31.9^{AB}	33.1^{A}	22.7^{C}	0.05	0.132	<0.001	0.652
Wheat, flattened	—	—	22.4	—	—	22.6	0.03	0.731	—	—
Soyabean meal	8.3^{B}	8.7^{A}	8.7^{A}	8.3^{B}	8.6^{A}	8.7^{A}	0.01	0.957	<0.001	0.827
Sunflower seed oil[4]	—	—	5.0	—	—	5.7	0.02	0.053	—	—
Fish oil[5]	—	2.1	—	—	2.3	—	0.01	0.127	—	—
Minerals and vitamins[6]	0.8^{B}	0.9^{A}	0.9^{A}	0.8^{B}	0.9^{A}	0.9^{A}	0.01	0.317	<0.001	0.773
Chemical composition, % of DM										
OM	92.7^{F}	92.9^{E}	94.5^{B}	93.1^{D}	93.3^{C}	95.0^{A}	0.05	<0.001	<0.001	0.746
CP	14.3^{AB}	14.0^{B}	13.1^{C}	14.4^{A}	14.2^{AB}	13.2^{C}	0.08	0.007	<0.001	0.822
NDF	36.4^{A}	35.0^{B}	29.7^{C}	36.6^{A}	34.8^{B}	29.5^{C}	0.43	0.752	<0.001	0.860
ADF	20.2^{A}	19.2^{B}	14.8^{C}	20.3^{A}	19.1^{B}	14.6^{C}	0.26	0.634	<0.001	0.812
Starch	23.0^{B}	23.6^{B}	32.4^{A}	23.3^{B}	24.1^{B}	32.6^{A}	0.50	0.210	<0.001	0.890
Ether extract	2.4^{c}	4.4^{b}	7.1^{a}	2.2^{c}	4.5^{b}	7.5^{a}	0.21	0.337	<0.001	0.067

$^{A–F}$Means within a row with different superscripts differ significantly ($P < 0.05$) due to the effect of diet or species × diet.

$^{a–c}$Means within a row with different superscripts indicate a tendency to differ ($P < 0.10$) due to the effect of species × diet.

[1]Control = no oil supplementation; FO = supplemented with fish oil; SOS = supplemented with sunflower seed oil plus additional starch from flattened wheat.

[2]SED = standard error of the difference.

[3]Probability of significant effects due to species (Sp), experimental diet (D), and interaction (Sp × D).

[4]Sunflower seed oil (Auvergne Trituration, Lezoux, France) contained (g/kg of FA): 16:0 (63.3), 18:0 (31.7), *cis*-9 18:1 (255.4), 18:2n-6 (630.2), 18:3n-3 (1.5), 20:0 (2.2), 22:0 (6.5), and 24:0 (2.6).

[5]Anchovy and menhaden oil (SA Daudruy Van Cauwenberghe & Fils, Dunkerque, France) contained (g/kg of FA): 12:0 (2.2), 14:0 (93.5), 15:0 (5.7), 16:0 (198.5), *cis*-9 16:1 (87.3), 17:0 (5.0), 18:0 (37.0), *cis*-9 18:1 (91.8), *cis*-11 18:1 (33.4), 18:2n-6 (16.2), 18:3n-6 (2.5), 18:3n-3 (13.6), 20:0 (4.4), *cis*-11 20:1 (1.6), 20:2n-6 (1.3), 20:3n-6 (1.6), 20:4n-6 (12.4), 20:5n-3 (183.4), 22:0 (1.2), 22:5n-3 (19.0), 22:6n-3 (76.7), 24:0 (2.0), and *cis*-15 24:1 (2.7).

[6]Mineral-vitamin mix (Groupe Centre-Lait, Aurillac, France) declared as containing (g/kg of premix): Ca (200), P (25), Mg (45), Na (35), Zn (6), Mn (3.5), and Cu (1.3).

Table 2. Effect of dietary supplements of fish oil or sunflower oil and starch on intake, milk yield, milk composition, and energy and protein balance in cows and goats[1]

	Cows			Goats				*P*-value[3]		
Item	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
DMI, kg/d	24.00^{A}	20.73^{B}	19.97^{B}	2.49^{C}	2.24^{C}	2.17^{C}	0.572	<0.001	<0.001	<0.001
DMI, g/kg of BW per day	39.1^{AB}	34.6^{BC}	32.8^{C}	43.3^{A}	39.0^{AB}	36.8^{BC}	1.85	0.007	<0.001	0.971
Fatty acid intake, g/d										
14:0	1.7^{C}	40.7^{A}	1.8^{C}	0.2^{D}	4.8^{B}	0.2^{D}	0.05	<0.001	<0.001	<0.001
16:0	85.4^{C}	156.4^{A}	126.9^{B}	9.2^{E}	18.0^{D}	14.5^{D}	1.92	<0.001	<0.001	<0.001
cis-9 16:1	1.0^{D}	37.5^{A}	1.7^{C}	0.1^{F}	4.4^{B}	0.2^{E}	0.03	<0.001	<0.001	<0.001
18:0	11.2^{C}	25.2^{B}	38.6^{A}	1.2^{F}	2.9^{E}	4.5^{D}	0.25	<0.001	<0.001	<0.001
cis-9 18:1	102.7^{C}	128.9^{B}	325.5^{A}	11.4^{E}	15.0^{E}	38.0^{D}	2.32	<0.001	<0.001	<0.001
cis-11 18:1	3.9^{B}	17.5^{A}	3.3^{C}	0.4^{E}	2.1^{D}	0.4^{E}	0.09	<0.001	<0.001	<0.001
18:2n-6	215.1^{B}	195.1^{C}	799.5^{A}	23.9^{E}	22.7^{E}	93.7^{D}	5.13	<0.001	<0.001	<0.001
18:3n-3	54.6^{A}	50.2^{B}	29.2^{C}	5.5^{D}	5.4^{D}	3.1^{E}	1.10	<0.001	<0.001	<0.001
20:5n-3	—	77.1	—	—	9.1	—	0.02	<0.001	—	—
22:5n-3	—	8.0	—	—	0.9	—	<0.01	<0.001	—	—
22:6n-3	—	32.2	—	—	3.8	—	0.01	<0.001	—	—
Total fatty acids	491.9^{C}	845.0^{B}	1,352.4^{A}	53.5^{F}	98.1^{E}	157.6^{D}	11.05	<0.001	<0.001	<0.001
Yield										
Milk, kg/d	29.82	30.31	29.57	2.46	2.45	2.46	1.015	<0.001	0.801	0.789
Fat, g/d	992^{A}	706^{B}	673^{B}	77^{C}	62^{C}	74^{C}	43.0	<0.001	<0.001	<0.001
Protein, g/d	833	839	875	81	82	86	32.0	<0.001	0.242	0.393
Lactose, g/d	1,513	1,525	1,491	120	126	129	53.9	<0.001	0.878	0.809
Yield										
Milk, g/d per kg of BW	48.50	50.75	48.25	42.39	42.01	43.63	3.577	0.052	0.814	0.366
Fat, g/d per kg of BW	1.611^{A}	1.174^{BC}	1.102^{BC}	1.339^{AB}	1.042^{C}	1.314^{ABC}	0.1417	0.574	<0.001	0.004
Σ <C16, mmol/d and per kg of BW	2.526^{ab}	1.680^{c}	1.117^{d}	2.762^{a}	2.194^{bc}	1.965^{bc}	0.2610	0.026	<0.001	0.075
Σ C16, mmol/d and per kg of BW	1.925^{A}	1.268^{BC}	0.882^{D}	1.329^{B}	1.022^{BCD}	0.877^{CD}	0.1466	0.023	<0.001	0.002
Σ >C16, mmol/d and per kg of BW	2.148^{ABC}	1.804^{BCD}	2.252^{AB}	1.584^{CD}	1.215^{D}	2.469^{A}	0.2078	0.062	<0.001	0.001
Concentration, g/kg										
Fat	33.4^{A}	23.4^{BC}	22.9^{C}	31.1^{A}	24.7^{C}	29.0^{AB}	1.88	0.294	<0.001	<0.001
Protein	28.0^{BC}	27.7^{C}	29.6^{B}	33.7^{A}	33.9^{A}	34.6^{A}	1.01	<0.001	0.004	0.359
Lactose	50.8^{AB}	50.4^{AB}	50.4^{AB}	49.3^{B}	50.7^{AB}	51.6^{A}	0.81	0.969	0.062	0.003
Energy balance,[4] %	115	114	127	130	133	133	6.3	0.008	0.080	0.212
Protein balance,[4] %	155^{AB}	148^{ABC}	130^{C}	167^{A}	162^{A}	132^{BC}	8.4	0.177	<0.001	0.379

$^{A–F}$Means within a row with different superscripts differ significantly ($P < 0.05$) due to the effect of diet or species × diet.

$^{a–d}$Means within a row with different superscripts indicate a tendency to differ ($P < 0.10$) due to the effect of species × diet.

[1]Control = no oil supplementation; FO = supplemented with fish oil; SOS = supplemented with sunflower seed oil plus additional starch from flattened wheat.

[2]SED = standard error of the difference.

[3]Probability of significant effects due to species (Sp), experimental diet (D), and interaction (Sp × D).

[4]Calculated according to INRA (2007).

Table 3. Messenger RNA relative abundance of genes involved in lipid metabolism in the mammary tissue of cows and goats fed diets supplemented with fish oil or sunflower oil and starch (arbitrary units determined as the abundance relative to the geometric mean of *RPLP0*, *UXT2*, and *EIF3K* mRNA and $\log_2$ transformed)[1]

	Cows			Goats				*P*-value[3]		
Pathway and genes	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
Lipid metabolism										
ACACA	10.18	10.17	10.04	9.90	9.09	9.96	0.419	0.054	0.278	0.181
FASN	4.79	4.48	4.69	3.57	3.41	3.88	0.475	0.001	0.545	0.814
G6PD	9.55^{BC}	9.54^{BC}	9.32^{C}	10.79^{A}	10.16^{B}	10.96^{A}	0.229	<0.001	0.081	0.006
CD36	7.96	7.90	8.04	8.23	8.06	8.17	0.369	0.452	0.832	0.952
FABP3	10.85	11.00	11.12	10.53	10.32	10.82	0.389	0.074	0.428	0.711
GPAM	4.38	4.52	4.72	6.55	6.39	6.80	0.511	<0.001	0.563	0.897
FADS1	11.30	11.08	11.44	8.24	7.51	8.15	0.377	<0.001	0.093	0.608
SCD1	10.07	10.06	10.08	9.08	8.75	8.95	0.447	0.001	0.813	0.836
SCD5	9.82	9.86	9.81	10.36	10.40	10.20	0.221	0.002	0.688	0.830
SLC2A1	10.53	10.35	10.33	10.40	10.35	10.40	0.198	0.880	0.627	0.720
GPD1	11.34	11.17	11.23	8.92	8.61	9.18	0.290	<0.001	0.241	0.394
XDH	9.76	9.51	9.79	11.37	11.27	11.55	0.302	<0.001	0.330	0.907
Milk protein										
LALBA	11.10	11.03	11.23	3.79	3.82	4.24	0.458	<0.001	0.510	0.859
CSN2	9.74	9.67	9.73	10.90	11.02	11.20	0.359	<0.001	0.800	0.813
Transcription factor										
SREBF1	11.05^{a}	10.63^{ab}	10.54^{ab}	9.97^{bc}	9.32^{c}	10.26^{ab}	0.352	<0.001	0.069	0.084
LXRA	10.78^{A}	10.33^{AB}	10.37^{AB}	10.12^{B}	10.14^{B}	10.28^{AB}	0.191	0.038	0.142	0.025
PPARA	16.91	16.97	16.99	16.22	16.92	15.93	0.426	0.031	0.198	0.197
PPARG1	10.94^{A}	10.70^{A}	10.72^{A}	9.85^{B}	9.82^{B}	10.33^{AB}	0.220	<0.001	0.162	0.038
SP1	10.04^{bc}	9.98^{bc}	9.87^{c}	10.45^{ab}	10.08^{bc}	10.57^{a}	0.201	0.001	0.212	0.087

[A–C]Means within a row with different superscripts differ significantly ($P < 0.05$) due to the effect of species × diet.

[a–c]Means (n = 12 for cows; n = 14 for goats) within a row with different superscripts indicate a tendency to differ ($P < 0.10$) due to the effect of species × diet.

[1]Control = no oil supplementation; FO = supplemented with fish oil; SOS = supplemented with sunflower seed oil plus additional starch from flattened wheat.

[2]SED = standard error of the difference.

[3]Probability of significant effects due to species (Sp), experimental diet (D), and interaction (Sp × D).

Table 4. Enzyme activity (nmol/min per milligram of protein) in the mammary tissue of cows fed diets supplemented with fish oil or sunflower oil and starch[1]

Item	Diet			SED[2]	*P*-value[3]
	Control	FO	SOS		
Fatty acid synthase	15.82	15.38	16.15	2.600	0.957
Malic enzyme	7.55[b]	10.5[a]	8.99[ab]	1.257	0.091
Glucose-6-phosphate dehydrogenase	74.45	72.44	70.77	6.487	0.853
Glycerol-3-phosphate dehydrogenase	409.2	454.6	442.3	43.36	0.565

[a,b]Means (n = 12) within a row with different superscripts indicate a tendency to differ ($P < 0.10$) due to the effect of diet.

[1]Control = no oil supplementation; FO = supplemented with fish oil; SOS = supplemented with sunflower seed oil plus additional starch from flattened wheat.

[2]SED = standard error of the difference.

[3]Probability of significant effects due to experimental diet.

Milk Fat Production and Composition

The data on animal performance and milk production and composition have been extensively reported and discussed in Toral et al. (2015). This direct comparison of cow and goat performance and responses to the FO and SOS treatments (with mean milk fat contents of 3.34 vs. 3.11 for the control, 2.34 vs. 2.47 for FO, and 2.29 vs. 2.90 for SOS in cows and goats, respectively; Toral et al., 2015) has confirmed interspecies differences in mammary lipogenesis, which were explored in the present study. Similarly, the observed dramatic decrease in the milk output of FA synthesized de novo (<C16) in cows fed SOS or FO, together with no changes in longer chain FA taken up from the blood (>C16; Table 2), is in line with characterized MFD (Bauman and Griinari, 2003; Shingfield and Griinari, 2007). In goats, an increase in the output of long-chain FA in the SOS treatment compensated for the decrease in short- and medium-chain FA, allowing milk fat secretion to be maintained, whereas an FO-induced decrease in milk fat content in goats was associated with a limited but significant decrease in the output of all FA, including long-chain FA, when expressed per kilogram of BW (Table 2). The data outline species specificities in the regulation of mammary lipogenesis response to the SOS and FO treatments. Among the mechanisms involved in this regulation and in the differences of response among species, our previous article (Toral et al., 2016) focused on ruminal aspects, demonstrating that main alterations due to SOS diet (i.e., the *trans*-10 shift and related increases in *trans*-10,*cis*-12 CLA) were more pronounced in cows than in goats, whereas changes linked to FO-induced MFD (e.g., decreases in 18:0 and increases in total *trans*-18:1 accumulation) were similar in caprines. However, the underlying molecular mechanisms occurring in the mammary tissue are still poorly understood and need to be further explored, which was the aim of the present study.

Mammary Metabolism

Species Specificities. A direct comparison of mRNA abundance of the 19 genes studied for cows and goats fed similar diets provided clear evidence of interspecies differences for 15 of the genes involved in milk component synthesis, which suggested differences in mammary metabolism between ruminant species (in an interaction with the diet for 3 of them). Indeed, for 12 of the genes, differences were observed among the species regardless of the dietary treatment. First, a higher mRNA abundance of 5 genes involved in lipid metabolism (*FASN, FADS1, GPD1, SREBF1,* and *PPARA*; Table 3) in cows compared with goats would suggest a higher transcription rate or lower degradation rate of the mRNA for these genes in cows compared with goats, but this was not related to milk fat content (Table 3). Furthermore, conversely to the *FASN* mRNA abundance, the FAS enzyme activity (Tables 5 and 6) is much higher in goats than in cows, which is in line with previous data (Bernard et al., 2013b). These observations are also in accordance with previous work reporting a higher concentration of the sum C6 to C14 in milk from goats than from cows, suggesting a proportionately higher de novo synthesis of these FA in goats (Chilliard et al., 2003). Second, a higher mRNA abundance of 3 genes involved in lipid metabolism (*XDH, GPAM,* and *SP1*) was observed in goats compared with cows. A previous study in goats (Bernard et al., 2012) demonstrated a positive association between mammary *XDH* and the gene involved in the esterification of FA to glycerol, which may suggest a shared regulation of the esterification pathway and of the proteins of the milk fat globule membrane, as observed in the present study. Third, whereas *LALBA* mRNA abundance was higher for cows compared with goats despite comparable lactose concentrations (Table 2), the opposite was observed for *CSN2* mRNA, which may be related to the milk protein content observed in

Table 5. Enzyme activity (nmol/min per milligram of protein) in the mammary tissue of cows and goats fed diets supplemented with fish oil or sunflower oil and starch[1]

Item	Cows			Goats			SED[2]	*P*-value[3]		
	Control	FO	SOS	Control	FO	SOS		Sp	D	Sp × D
Fatty acid synthase	10.42	10.11	11.41	130.0	84.6	122.0	36.24	<0.001	0.609	0.627
Malic enzyme	8.83	12.52	9.98	5.67	4.00	5.04	2.104	<0.001	0.742	0.158
Glucose-6-phosphate dehydrogenase	75.3	72.7	77.8	283.1	169.2	233.2	56.19	<0.001	0.355	0.320
Glycerol-3-phosphate dehydrogenase	435.0	474.8	495.5	87.5	44.9	80.4	64.93	<0.001	0.784	0.612

[1]Control = no oil supplementation; FO = supplemented with fish oil; SOS = supplemented with sunflower seed oil plus additional starch from flattened wheat.
[2]SED = standard error of the difference.
[3]Probability of significant effects due to species (Sp), experimental diet (D), and interaction (Sp × D).

this study (2.84 vs. 3.41 g/100 g; mean of the 3 dietary treatments for cows and goats, respectively). However, these putative associations between mRNA abundance and the observed production data must be considered with caution because of posttranscriptional events that may occur. Last, there was a higher *SCD1* mRNA abundance in cows compared with goats, whereas the opposite was observed for the *SCD5* gene. These results, together with much higher *cis*-9 10:1/10:0, *cis*-9 14:1/14:0, and *cis*-9 16:1/16:0 ratios in milk of cows compared with goats (Table 2) suggests a more extensive Δ^9-desaturation of FA in the bovine than in the caprine mammary gland that could be attributable to SCD1 protein activity. Accordingly, a previous comparative study of mammary lipid metabolism in cows and goats revealed a higher expression of *SCD1* in cows, whereas the data on *SCD5* from both species were not reported (Bernard et al., 2013b). Indeed, the respective role of SCD1 and SCD5 in the mammary gland has not yet been clearly specified even though few studies suggested both different expression and regulation for these 2 isoforms (Bernard et al., 2013a).

The activity of the corresponding proteins of the *G6PD* and *GPD1* genes were measured in goats and cows, and conversely to *FASN*, similar rankings of mRNA abundance and protein activity (G6PDH and G3PDH; Table 5) among species were observed. These data suggest differential posttranscriptional regulation of *FASN* among species due to (1) higher translation repression or degradation of mRNA in cows, which affects *FASN* protein synthesis and mRNA stability (Semenkovich et al., 1993) and may involve microRNA such as miR-145 (Wang et al., 2017) and miR-24 (Wang et al., 2015) that target *FASN* mRNA; (2) different ubiquitination of lysine or phosphorylation in sites of the protein sequence affecting protein activity (Wakil et al., 1989; Jensen-Urstad and Semenkovich, 2012); or (3) other mechanisms. In addition to the multifunctional enzyme FAS, the biosynthesis of de novo FA requires considerable amounts of reducing equivalents in the form of NADPH. In ruminants, NADPH is mainly generated through the pentose phosphate and citrate dehydrogenase pathways (Moore and Christie, 1981), which involves G6PDH and isocitrate dehydrogenase enzymes, whereas malic enzyme contribution is minor. Both FAS and G6PDH activities were higher in goats compared with cows, which is in line with a higher concentration of the sum C6 to C14 in this species, whereas malic enzyme activity was higher in cows (Table 4). In other respects, G3PDH activity is much higher in cows compared with goats, which was in line with the abundance of the encoding mRNA but in disagreement with *GPAM*. These findings contribute to a

Table 6. Pearson correlation coefficients (>+0.40 and <−0.40) between mRNA abundance (arbitrary units) and milk fat content and secretion of specific fatty acids in milk (g/d of fatty acids) from goats fed diets supplemented with fish oil or sunflower oil and starch[1,2]

Item	Fat content	4:0	8:0	16:0	*cis*-11 16:1	18:0	*cis*-11 18:1	*trans*-9,*trans*-11 CLA	18:2n-6	18:3n-3	22:5n-3
G6PD					−0.405**						−0.445**
GPD1					−0.405**						
XDH									+0.451**		
LXRA[3]	−0.505**	−0.507***	−0.601***	−0.579***	−0.458**		−0.549***			−0.558***	
SREBF1											−0.401*
PPARG1						+0.401*		+0.454**			
SP1[4]											−0.413**

[1]Control = no oil supplementation; FO = supplemented with fish oil; SOS = supplemented with sunflower oil plus additional starch from flattened wheat.
[2]Relationships were derived using 42 measurements obtained from 14 animals. Signs indicate the effect of the variable on the predictor.
[3]Significant correlation also with C6, C10, C12, C14, and *cis*-9 16:1.
[4]Significant correlation also with 20:5n-3 and 22:6n-3.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

better understanding of the physiology of lipogenesis among species that would need further investigation.

The results of the study also demonstrated significant correlations between most of the studied genes. Indeed, in both species, *LXRA* and *PPARA* were significantly and negatively associated ($-0.70 < r < -0.32$, $n = 21$) with almost all the other genes, whereas significant and positive associations ($+0.32 < r < +0.87$, $n = 22$) were observed for *SREBF1* and *SP1*, respectively, suggesting a putative implication in the repression and induction of the genes by these transcription factors, which is in line with several studies in bovines (Bionaz and Loor, 2008; Kadegowda et al., 2009; Oppi-Williams et al., 2013) and caprines (Shi et al., 2013; Xu et al., 2016; Yao et al., 2016) that reported crucial roles of these transcription factors on lipogenic gene expression. Such relationships might result from shared regulation by the same transcription factor or from concomitant regulation by different transcription factors (Harvatine and Bauman, 2006) for these genes, regulation involving microRNA (Lynn, 2009), or other mechanisms.

Responses to the Starch-Rich Diet Plus Sunflower Oil and to FO Supplements. Even though the supplements of sunflower oil and wheat starch dramatically lowered (−31%; Toral et al., 2015) the milk fat content in cows without affecting milk yield, little or no variation in mammary mRNA and enzyme activities was observed in the present study. This absence of variation in candidate gene expression and enzyme activity is not consistent with the reported decrease in mRNA or the activity of lipogenic enzymes in response to diets containing high proportions of concentrate or plant oils in cows (Piperova et al., 2000) showing considerable milk fat decreases (−43%). Similarly, the supplements of FO in the present study lowered milk fat content in cows (−31%; Toral et al., 2015) and had no or little effect on mammary mRNA and enzyme activities. These data are not consistent with the reported decreasing effects of diets containing FO (Ahnadi et al., 2002; Harvatine and Bauman, 2006) or the combination of plant oil and algae (Angulo et al., 2012) on mammary mRNA abundance of lipogenic genes in cows and milk fat content (−34% in Ahnadi et al., 2002; −31% in Harvatine and Bauman, 2006; and −39% in Angulo et al., 2012).

These differences in the response of gene expression between previous studies in cows and the present one, despite similar effects on milk fat content, may be partly attributable to methodological differences, including the sampling procedure, the mRNA abundance measurement, or the time at which mammary tissue sampling was performed relative to concentrate distribution and milking. Regarding the methodology for the measurement of mRNA abundance on mammary tissue

collected by biopsy, the same method was used in the present study in cows and in Angulo et al. (2012) on tissue collected at slaughter from cows fed a combination of plant oil and algae, where effects on mammary mRNA abundance were reported. When considering the time of sampling relative to milking and the last meal, in most of the abovementioned studies this information was not available except in Harvatine and Bauman (2006), which specified that cow mammary biopsies were collected using a Bard biopsy system at 1 to 3 h after milking and with diets consumed ad libitum, suggesting free access to the TMR. Conversely, in the present study, mammary biopsies were obtained before the morning milking and feeding, which was at least 16 h after the evening meal and milking. Thus, a possible short-term regulation of mRNA synthesis by nutrient supply and milking cannot be ruled out (Chen et al., 2008; Wall and McFadden, 2010), which could contribute to the explanation of the observed dietary response of mRNA in Harvatine and Bauman (2006) and an absence of response in the present study. In *Drosophila*, which was used as a model for studying nutrient-dependent genes by a transcriptomic approach because many aspects of the basic logic of nutrient signaling should be conserved among species, up- or downregulation of most of the genes in response to nutrients for 4 to 12 h was observed (Zinke et al., 2002). Moreover, under a given physiologic condition, mRNA can display a wide range of stability, with a half-life ranging from 10 to 15 min to several hours in mammalian cells (Chen et al., 2008) depending on their role (Sharova et al., 2009). Thus, each mRNA has its specific stability, which can change in response to a variety of extracellular stimuli such as nutrient supply. Finally, proteins are on average 5 times more stable (median half-life of 46 h) than mRNA (9 h) and span a bigger range (Schwanhäusser et al., 2011). From the above information on both the delay of the response to nutrient stimuli and mRNA half-time and our data on the lack of dietary response of mRNA abundance from a mammary biopsy at a given point (16 h after the evening meal), we can speculate that the time of collection of the mammary samples could interact with the effects of diets on the mRNA abundance of genes involved in lipid and protein metabolism in ruminant mammary tissue. However, this needs to be demonstrated by ad hoc studies because the high rumen retention time of the feed particles (>30 h) in cows (Grandl et al., 2016) and goats (Leite et al., 2015) maintains a more constant nutrient delivery to tissues, including the mammary gland, compared with nonruminant species. Nevertheless, the limitation of mammary expression of the genes implicated in milk synthesis due to the accumulation of milk in the mammary epithelial cell still must be considered and could be another mechanism contributing in part to the absence of changes in gene expression in response to the dietary treatments (Wall and McFadden, 2010).

Pearson correlation performed between the 19 mRNA abundances and the milk yields of individual FA revealed 15 significant correlations with $r > +0.40$ or $r < -0.40$ in goats (Table 6), whereas no correlations were observed within this rank in cows. In goats, the 3 positive interindividual relationships between mRNA abundance and the milk output of FA (between *PPARG1* and 18:0 and *trans*-9,*trans*-11 CLA and between *XDH* and 18:2n-6) and the 12 negative relationships (7 between *LXRA* and 4:0, 8:0, 16:0, *cis*-11 16:1, *cis*-11 18:1, and 18:3n-3; 2 between *G6PD* and *cis*-11 16:1 and 22:6n-3; 1 between *GPD1* and *cis*-11 16:1; 2 between *SREBF1* and *SP1*, respectively, with 22:6n-3) would need further investigation to decipher the biological implications of these associations. In line with the present data on lipogenic genes, negative relationships between milk fat content (g/kg) and the concentrations of *cis*-11 16:1 and *cis*-11 18:1 and a positive relationship between milk fat content and 18:0 was reported in goats in the present study (Toral et al., 2015) as well as in Bernard et al. (2015a) in goats fed grass hay-based diets supplemented or not with either extruded linseeds or extruded linseeds and FO. In the present study, it was specifically noted that there was a negative correlation between *LXRA* and milk fat content and individual short- and medium-chain FA yield and a positive correlation between *PPARG1* and 18:0 yield. Further studies on the regulation of these transcription factors are necessary to elucidate the mechanisms underlying these relationships and verify whether they imply causation.

The effects of the dietary treatments on the activity of lipogenic enzymes could be absent because the in vitro activity assay was done in optimal conditions (pH, substrate, cofactors), which differed from the in vivo conditions (Bernard et al., 2008). However, other enzymes and mechanisms such as nutrient flow and hormonal status may intervene in the dietary regulation of mammary lipid synthesis.

A general view of the present and previous (Toral et al., 2015, 2016) studies for the direct comparison of cow and goat performance and milk FA composition responses to the FO and SOS treatments suggests interspecies differences in mammary lipogenesis, which may be attributable to interspecies differences in the rumen biosynthesis of BH intermediates known for their antilipogenic effects or their mammary metabolism specificities. Furthermore, the main alterations in the ruminal BH pathways potentially responsible for MFD in the SOS diet (i.e., the shift from *trans*-11 to

trans-10 18:1 and related increases in *trans*-10,*cis*-12 18:2) tended to be more pronounced in cows, which was consistent with the associated MFD only in this species. Otherwise, in the caprine, rumen fluid changes linked to FO (e.g., decreases in 18:0 and increases in total *trans*-18:1) were stronger compared with those in the bovine, which may explain the unexpected negative effects of FO on milk fat content (although less marked than that in bovines). Elsewhere, the changes in milk fat content and FA secretion were not associated with alterations in the mammary expression or the activity of 19 proteins involved in the major lipogenic pathways. This absence of observed variation may be due to regulation at other levels (posttranscriptional or posttranslational) for these genes and related enzymes or related to the time of sampling of the mammary tissue relative to the previous meal and milking or both. Otherwise, strong species specificities in the abundance of mRNA encoding for genes involved in lipid metabolism or enzyme activities in the mammary gland were observed, providing evidence that the major mammary lipogenic pathways differ between the caprine and bovine. The biological significance of these species differences remains to be unraveled.

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