# Effect of diets supplemented with starch and corn oil, marine algae, or hydrogenated palm oil on mammary lipogenic gene expression in cows and goats: A comparative study

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# Effect of diets supplemented with starch and corn oil, marine algae, or hydrogenated palm oil on mammary lipogenic gene expression in cows and goats: A comparative study

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## ABSTRACT

A direct comparison of cow and goat performance and milk fatty acid (FA) responses to diets that either induce milk fat depression or increase milk fat content in cows suggests species-specific regulation of lipid metabolism, including mammary lipogenesis. This experiment was conducted to highlight potential mechanisms responsible for the differences in mammary lipogenesis due to diet and ruminant species. Twelve Holstein cows and 12 Alpine goats were fed a basal diet containing no additional lipid (CTL) or a similar diet supplemented with corn oil [5% dry matter intake (DMI)] and wheat starch (COS), marine algae powder (MAP; 1.5% DMI), or hydrogenated palm oil (HPO; 3% DMI), according to a 4 × 4 Latin square design with 28-d experimental periods. Milk yield, milk composition, FA profile, and secretions were measured. On d 27 of each experimental period, the mRNA abundance of 21 genes involved in lipid metabolism or enzyme activities or both were measured in mammary tissue sampled by biopsy. The results showed significant differences in the milk fat response of cows and goats to the dietary treatments. In cows, fat content was lowered by COS (−45%) and MAP (−22%) and increased by HPO (+13%) compared with CTL, and in goats only MAP had an effect compared with CTL, with a decrease of 15%. In both species, COS and MAP lowered the yields (mmol/d per kilogram of body weight) of <C16 and C16 FA. With COS, this decrease was compensated by an increase of >C16 FA in goats but not in cows, and the >C16 FA yield decreased with MAP in both species. Supplementation of HPO increased the yield of milk C16 FA (mmol/d per kilogram of body weight) in cows. These variations in milk fat content and FA secretion were not associated with modifications in the mammary expression of 21 genes involved in major lipid pathways, except for 3 transcription factors: *PPARA*, *INSIG1*, and *SP1*. This absence of large changes might be due to post-transcriptional regulation of these genes and related to the time of sampling of the mammary tissue relative to the previous meal and milking or to differences in the availability of substrate for the corresponding proteins. However, the abundance of 14 mRNA among the 21 encoding for genes studied in the mammary gland was significantly different among species, with 5 more abundant in cows (*FADS3*, *ACSL1*, *PPARA*, *LXRA*, and *PPARG1*) and 10 more abundant in goats (*FASN*, *CD36*, *FABP3*, *LPL*, *GPAM*, *LPIN1*, *CSN2*, *MFGE8*, and *INSIG1*). These species specificities of mammary lipid metabolism require further investigation.

**Key words:** ruminant species, lipid supplement, mammary gland, lipogenic gene expression, milk fat plasticity

## INTRODUCTION

The milk fat content and composition of ruminants are determinants of the feed efficiency of animals and of the nutritional quality for the consumer (Chilliard et al., 2007). Thus, increased understanding of the mechanisms involved in milk fat synthesis is a prerequisite to modulate milk fat content and composition. Among breeding factors, nutrition is a rapid and efficient tool to modulate milk fat content and composition; in particular, the addition of lipid supplements in ruminant diets has been widely used these last decades to improve milk fatty acid (**FA**) composition. In cows, under certain dietary conditions, such as diets rich in starch and supplemented with plant oils or diets supplemented with marine lipids, milk fat depression (**MFD**) occurs (Bauman and Griinari, 2001). To explain this phenomenon, in diets rich in starch and plant oils the biohydrogenation (**BH**) theory prevails, as these diets modify the BH pathways of PUFA in the rumen, with a consequent shift from the *trans*-11 to *trans*-10 isomer formation, with some of them having antilipogenic ef-

fects, particularly *trans*-10,*cis*-12 CLA (Bauman and Griinari, 2003). For marine lipids, MFD has been attributed to an inhibition of the last step of rumen BH pathways that conduct to a concomitant 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 (Shingfield and Griinari, 2007), which might increase milk fat melting point and impair fat secretion. However, these theories alone do not explain the MFD, suggesting that other mechanisms are involved.

Moreover, indirect comparison studies suggest that MFD is not commonly observed in small ruminant species, particularly in goats under dietary conditions similar to those that induce MFD in cows (Chilliard et al., 2003; Shingfield et al., 2010). To confirm these findings, a first direct comparison between dairy cows and goats revealed that the caprine is less sensitive to the 2 types of diets known to induce MFD (high starch and plant oil or fish oil diets) in the bovine (Toral et al., 2015). A more complete comparison study was recently conducted with dairy cows and goats fed various lipid supplements known to induce MFD or, conversely, to increase fat content in the bovine and for which the effects in goats are absent or unknown (Fougère et al., 2018). That study revealed relevant interspecies differences and species-by-diet interactions, including (1) high-starch diets containing plant oils or addition of marine algae in the diet induced MFD in cows and had, respectively, no or lesser effect in goats, and (2) a hydrogenated palm oil diet induced a milk fat increase in cows and not in goats. The reasons for these differential lipogenic responses between 2 closely related ruminant species are not well understood, but based on indirect and on the unique direct comparisons of milk FA composition, both ruminal BH and mammary lipid metabolism (Shingfield et al., 2010; Toral et al., 2016; Bernard et al., 2017) could be implicated. The aim of the present study was to provide further insight into the mechanisms regulating mammary lipid metabolism in ruminants to help to control milk fat secretion in ruminants and find a way to reduce the MFD observed in cows under specific dietary conditions. Indeed, milk fat synthesis is under the control of a set of genes whose alteration in expression could partly explain the diet-induced MFD or fat augmentation, with underlying mechanisms that might differ depending on dietary conditions or animal species. Moreover, previous studies in cows under MFD outlined differences in the responses of mammary gene expression as either decreases (Piperova et al., 2000; Ahnadi et al., 2002; Harvatine and Bauman, 2006; Angulo et al., 2012) or no effect (Bernard et al., 2017). Thereby, our direct comparative study on dairy cows and goats was designed to test the following hypotheses: (1) mammary genes expression underlying MFD induced by starch and plant oils or marine supplements differ; (2) diet-induced increases in fat yield could result from higher availability of mammary substrate for lipogenesis rather than modification of mammary gene expression; and (3) these mechanisms differ between ruminant species, with the goat less sensitive to changes induced by diet than the cow. To achieve this goal, cows and goats were fed a basal diet (**CTL**), a similar diet supplemented with corn oil and additional starch from wheat (**COS**), a diet supplemented with marine algae powder (**MAP**), or a diet supplemented with hydrogenated palm oil (**HPO**). Changes in animal performances, milk FA yields, plasma metabolites, expression of several genes involved in the major lipogenic pathways, and the activities of a few lipogenic enzymes were measured to deduce the potential mechanisms responsible for the differences in mammary lipogenic responses to diets and ruminant species.

## MATERIALS AND METHODS

### Animals, Experimental Design, Diets, and Management

The Auvergne Rhône-Alpes Ethics Committee for Experiments on Animals approved all experimental procedures (France; DGRI agreement APAF-IS#3277–2015121411432527 v5), which were compliant with the guidelines established by the European Union Directive 2010/63/EU (European Union, 2010). The details of the experimental design are described in Fougère et al. (2018). Briefly, 12 Holstein cows and 12 Alpine goats, all multiparous, nonpregnant and at a lactation stage of $86 \pm 24.9$ and $61 \pm 1.8$ DIM, respectively, were allocated to 1 of 4 groups (3 cows and 3 goats per group). Groups were balanced according to DIM, milk production, milk fat, and milk protein content in a replicated $4 \times 4$ Latin square design to test the effects of 4 treatments that were randomly assigned to each group over four 28-d experimental periods. All animals were offered a diet composed of grass hay ad libitum with concentrates containing no additional lipid (CTL), corn oil and wheat starch (COS), marine algae powder of *Schizochytrium* sp. (MAP), or hydrogenated palm oil (HPO). Formulation of experimental concentrates and chemical composition of concentrates and grassland hay were described in Fougère et al. (2018). In the COS, MAP, and HPO treatments, corn oil (5.0% total DMI), marine algae powder of *Schizochytrium* (1.5), and hydrogenated palm oil (3.0), respectively, were added to

the concentrate manually immediately before applying feed. Diets were offered as 2 equal meals at 0830 and 1600 h, starting with the concentrate (supplemented or not in lipids) distribution and followed by hay. Concentrate and hay refusals were weighed daily and used to adjust the amounts of feed offered the following day to maintain the targeted dietary forage-to-concentrate ratio (45:55 on a DM basis). The formulation, chemical composition, and FA profile of the concentrates and hay have been reported previously (Fougère et al., 2018). Corn oil, marine algae powder, and hydrogenated palm oil supplements were supplied at 920 and 111 g/d, 310 and 40 g/d, and 630 and 80 g/d in cows and goats, respectively. The animals had access to a constant supply of fresh water ad libitum and were milked at 0800 and 1500 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 (Fougère et al., 2018). The milk yields of individual animals were recorded over 6 milkings at 0800 and 1500 h on d 21, 22, and 24 of each of the 4 experimental periods. Simultaneously, milk samples were individually collected and treated with preservative (bronopol-B2; LIAL, Aurillac, France) to measure fat, protein, and lactose. Unpreserved milk samples were also collected over 2 consecutive milkings starting at 0800 h on d 24 of each experimental period and then stored at −20°C for analysis of FA composition (Fougère et al., 2018).

On d 27 of each experimental period, mammary tissue was collected under sterile conditions using a biopsy instrument (AgResearch Ruakura, Ruakura Agricultural Center, Hamilton, New Zealand), as previously described by Farr et al. (1996), for cows and the Acecut Gun (11 gauge × 11.5 cm; Mediapi, Bourges, France) for goats. Mammary biopsies were obtained 5 to 7 h after the morning milking and the morning feeding following the procedure previously described (Bernard et al., 2017). Approximately 600 and 30 mg of mammary tissue for cows and goats, respectively, were collected from a midpoint on a rear quarter, alternating from the 2 rear quarters of the udder (for the 4 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 (fourth period), the goats were integrated into a herd of dairy goats in the mid-mountain area of the region Auvergne-Rhône-Alpes and cows joined the herd of the experimental farm.

### RNA Isolation and Real-Time Reverse-Transcription PCR

Total RNA was prepared with the homogenization of approximately 120 mg of cow mammary tissue and 30 mg of goat mammary tissue in 1 and 0.35 mL of Trizol Reagent (Life Technologies, Saint Aubin, France), respectively, followed by isolation using a Pure Link RNA mini kit isolation system (Life Technologies). Potential contaminating genomic DNA was removed through a DNase treatment step (RNase-Free DNase Set #79254, Courtaboeuf, France). The RNA concentrations were determined by measuring absorbance at 230, 260, and 280 nm using a NanoDrop (ND-1000 spectrophotometer; NanoDrop, Labtech, Palaiseau, France). The RNA integrity was determined using a 2100 Bioanalyzer (Agilent Technologies, Massy, France) and was 8.2 (SD 0.53) and 8.3 (SD 0.38) on average for mammary RNA from cows and goats, respectively.

Using total RNA isolated from the mammary biopsy samples, reverse transcription was performed with 2 μg of purified total RNA using a High-Capacity RNA-to-cDNA kit (Ref. 4387406; Life Technologies) in a final volume of 20 μL. The samples were stored at −20°C.

The mRNA abundance of 21 candidate genes was measured via quantitative real-time reverse transcription PCR. Involved in de novo FA synthesis was FA synthase (*FASN*), whereas FA translocase (*CD36*), fatty acid binding protein 3, muscle and heart (*FABP3*) were involved in FA uptake and solute carrier family 2 (facilitated glucose transporter), with member 1 (*SLC2A1*) was involved in glucose uptake. Lipoprotein lipase (*LPL*) was involved in the uptake of FA from circulating triacylglycerol. Involved in FA desaturation were stearoyl-CoA desaturase 1 (*SCD1*), stearoyl-CoA desaturase 5 (*SCD5*), and fatty acid desaturase 3 (*FADS3*). Glycerol phosphate acyltransferase (*GPAM*) and phosphatidate phosphatase LPIN1 (*LPIN1*) were involved in triglyceride synthesis. Acyl-CoA Long Chain Family Member 1 (*ACSL1*) was involved in fatty acid activation and transport. β-Casein (*CSN2*) is a major protein in milk. Lactadherin (*MFGE8*) is a protein of milk fat globule membrane, and mechanis-

tic target of rapamycin kinase (*MTOR*) was involved in protein synthesis. Toll-like receptor 4 (*TLR4*) was involved in inflammation, 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*), Sp1 transcription factor (*SP1*), and insulin-induced gene 1 protein (*INSIG1*) were 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 arithmetic mean of 3 reference 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 transcription PCR using the StepOnePlus TM real-time PCR system and SYBR Green dye (Power SYBRGreen PCRMasterMix) or a fluorescent TaqMan probe (TaqManFast Universal PCR Master Mix, Life Technologies). Specific primers and probes were designed on a consensus cDNA fragment between species. Briefly, for SYBRGreen technology, after an initial denaturing step (95°C for 10 min), the PCR mixture was subjected to a 2-step cycle repeated 40 times consisting of denaturing for 15 s at 95°C and annealing for 45 s at 58, 60, or 62°C (depending of the primer pairs). Real-time PCR based on TaqMan probe technology was performed under the same conditions, but the annealing for primer pairs was always for 45 s at 60°C.

The PCR efficiency was 94.5% (SD 8.38) for the 21 target genes and 99.7% (SD 0.44) 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 reference genes to account for variations in RNA integrity. Relative mRNA abundance was calculated using the $2^{-\Delta\mathrm{CT}}$ method, where cycle threshold (CT) is the endpoint of real-time PCR analysis, and the delta CT is CT gene – CT arithmetic mean of the 3 reference genes.

### Enzyme Assays

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

### Statistical Analyses

The mRNA abundance data were subjected to ANOVA for a 4 × 4 Latin square design (Kaps and Lamberson, 2009) using the MIXED procedure in the SAS statistical software package (version 9.4, SAS Institute Inc., Cary, NC). The statistical model included the fixed effects of period, species (**Sp**), experimental diet (**D**), the interaction Sp × D, and the random effect of individual animal nested within species. For cows, enzyme activity data were subjected to ANOVA for a 4 × 4 Latin square design using the MIXED procedure in SAS. The statistical model included the fixed effects of period, Sp, D, the interaction Sp × D and the random effect of animal nested within treatment. 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 Tukey-Kramer's method; significance was declared at $P < 0.05$. $P$-values between >0.05 and ≤0.10 were interpreted as trending toward significance. Pearson correlation coefficients (r) were generated for associations between the abundance of mammary mRNA among themselves and the concentration of specific FA in the milk and plasma metabolites. Pearson correlation coefficients were computed using XLStat software (Version 2009.1.01, Addinsoft, Paris, France) and the correlation values were considered significant at $P < 0.05$.

### 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 [45:55, on a DM basis; see Fougère et al. (2018) for more details]. The inclusion of oil resulted in more ether extract in the COS, MAP, and HPO treatments than in the control (Table 1). The starch content in the COS treatment increased by approximately 29% compared with the control. By design, the inclusion of corn oil resulted in increased intake of *cis*-9 18:1 and 18:2n-6 in the COS diet, with 18:2n-6 the primary FA, whereas the addition of marine algae powder increased the intake of 14:0, 22:5n-3, and 22:6n-3 in the MAP treatment; the

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addition of hydrogenated palm oil increased the intake of 16:0 and 18:0 in the HPO treatment (Table 2).

### Animal Performance

The effects of the treatments on animal performance and milk composition are reported in Table 2, with more details in Fougère et al. (2018). The DMI per kilogram of BW was 50% higher ($P < 0.001$) in goats than in cows, and the milk yield per kilogram of BW was higher ($P = 0.002$, 38%) for goats than for cows (Table 2). Milk fat, protein, and lactose contents were similar between species in the controls. The daily yield of milk <C16, expressed as millimoles per kilogram of BW, was lower in cows than in goats fed the control treatment ($P < 0.001$), but no differences 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), with a mean decrease in COS of 15% compared with that of CTL. In cows, COS decreased the milk fat content by 45% compared with the CTL (Table 2), and MAP decreased the fat content by 22 and 15% in cows and

goats, respectively; moreover, HPO increased milk fat content in cows by 13%. In cows, protein content with COS increased by 7% compared with that in the CTL. In cows, MAP decreased lactose content by 5% (Table 2).

In cows, COS decreased the secretion (mmol/d and per kilogram of BW) of all fatty acid classes (<C16, C16, and >C16), whereas in goats this diet only decreased <C16 and C16 but increased >C16. In cows, MAP decreased secretion of C16 and >C16, which was also observed in goats in addition to that of the <C16. In cows, HPO only increased secretion of C16, which was not observed in goats (Table 2).

### Mammary Lipid Metabolism

Among the 21 mRNA encoding for genes involved in mammary metabolism, the abundance of 14 was significantly affected by species ($P < 0.05$), with a tendency ($P < 0.10$) for significance for *TLR4* and no significant effect ($P > 0.10$) for *SCD1*, *SCD5*, *MTOR*, *SLC2A1*, *SP1*, and *SREBF1* (Table 3). Of the 14 mRNA transcripts differentially expressed between species, 5 were more abundant in cows (*FADS3*, *ACSL1*, *PPARA*,

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

| Item | Cows | | | | Goats | | | | P-value[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | COS | MAP | HPO | Control | COS | MAP | HPO | SEM | Sp | D | Sp × D |
| Ingredient, % of DM | | | | | | | | | | | | |
| Grassland hay | 45.4 | 43.6 | 44.6 | 45.3 | 42.9 | 43.2 | 43.8 | 43.6 | 0.28 | 0.003 | 0.296 | 0.227 |
| Concentrate[3] | 54.6 | 51.4 | 53.9 | 51.8 | 57.1 | 51.6 | 54.7 | 53.4 | 0.27 | 0.002 | <0.001 | 0.137 |
| Lipid supplement[4] | — | 5.0 | 1.5 | 3.0 | — | 5.1 | 1.5 | 3.0 | 0.07 | 0.719 | <0.001 | 0.965 |
| Chemical composition, % of DM | | | | | | | | | | | | |
| OM | 92.2 | 93.3 | 91.7 | 92.0 | 92.2 | 93.3 | 91.7 | 92.0 | 0.01 | 0.773 | <0.001 | 0.955 |
| CP | 21.0 | 19.8 | 22.6 | 20.1 | 21.4 | 19.8 | 22.7 | 20.3 | 0.004 | 0.037 | <0.001 | 0.223 |
| NDF | 39.2 | 33.7 | 39.0 | 39.0 | 38.1 | 33.5 | 38.7 | 38.3 | 0.13 | 0.006 | <0.001 | 0.343 |
| ADF | 21.9[b] | 36.9[a] | 21.8[bc] | 21.9[b] | 21.3[c] | 36.8[a] | 21.6[bc] | 21.5[bc] | 0.06 | 0.002 | <0.001 | 0.069 |
| Starch | 19.9 | 26.1 | 18.6 | 17.4 | 20.8 | 26.2 | 18.8 | 18.0 | 0.11 | 0.003 | <0.001 | 0.247 |
| Ether extract | 1.9 | 6.7 | 2.5 | 4.8 | 1.9 | 6.8 | 2.5 | 4.8 | 0.65 | 0.660 | <0.001 | 0.972 |

[a–c]Means within a row not sharing a common superscript differ ($P < 0.10$) due to species by diet interactions.

[1]Control = basal diet containing no additional oil; COS = basal diet containing corn oil and wheat starch; MAP = basal diet containing marine algae powder; HPO = basal diet containing hydrogenated palm oil.

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

[3]Control concentrate (g/kg of DM): corn (532), soy (138), dehydrated alfalfa (275), molasses cane (37), dicalcium phosphate (2), carbonate flour (11), salt (3), mineral and vitamin complement (2); COS concentrate (g/kg of DM): wheat (395), corn (394), soy (150), molasses cane (35), dicalcium phosphate (2), carbonate flour (19), salt (3), mineral and vitamin complement (2); MAP concentrate (g/kg of DM): corn (518), soy (142), dehydrated alfalfa (283), molasses cane (38), dicalcium phosphate (2), carbonate flour (12), salt (3), mineral and vitamin complement (2); HPO concentrate (g/kg of DM): corn (500), soy (147), dehydrated alfalfa (294), molasses cane (39), dicalcium phosphate (2), carbonate flour (13), salt (3), mineral and vitamin complement (2).

[4]In COS: corn oil (Olvea, Saint Léonard, France) was added to the concentrate at 5% of total DMI and contained [g/kg of total fatty acid (FA)]: 16:0 (114), 18:0 (16.4), *cis*-9 18:1 (297), *cis*-11 18:1 (6.30), 18:2n-6 (535), 18:3n-3 (7.57), 20:0 (3.48), 22:0 (1.0), 24:0 (1.5), and total FA (1,000 g/kg). In MAP: marine algae powder (DSM, Basel, Switzerland) was added to the concentrate at 1.5% of total DMI and contained (g/kg of total FA): 12:0 (1.12), 14:0 (42.7), 15:0 (1.73), 16:0 (117), *cis*-9 16:1 (0.88), 17:0 (0.29), 18:0 (2.47), *cis*-9 18:1 (0.56), *cis*-11 18:1 (0.55), 18:2n-6 (0.07), 18:3n-3 (0.18), 20:0 (0.17), 20:3n-6 (2.18), 20:4n-6 (2.62), 22:0 (0.24), 22:5n-3 (2.58), 22:6n-3 (370), *cis*-15 24:1 (0.25), and total FA (717 g/kg). In HPO: hydrogenated palm oil (Provimi, Crevin, France) was added to the concentrate at 3% of total DMI and contained (g/kg of total FA): 12:0 (5.09), 14:0 (12.4), 15:0 (0.51), 16:0 (463), 17:0 (1.26), 18:0 (474), *cis*-9 18:1 (11.8), *cis*-11 18:1 (0.71), *cis*-9,*cis*-12 18:2 (0.78), 20:0 (3.39), 20:4 n-6 (3.58), and total FA (995 g/kg).

**Table 2.** Effect of dietary supplements of corn oil and starch or marine algae powder or hydrogenated palm oil on intake, milk yield, milk composition, and energy and protein balance in cows and goats[1]

| Item | Cows | | | | Goats | | | | SEM | P-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | COS | MAP | HPO | Control | COS | MAP | HPO | | Sp | D | Sp × D |
| DMI, kg/d | 22.27^A | 18.53^B | 21.30^A | 21.31^A | 2.55^C | 2.24^C | 2.47^C | 2.65^C | 0.393 | <0.001 | <0.001 | <0.001 |
| DMI, g/kg of BW per day | 32.96 | 27.12 | 31.58 | 31.68 | 47.21 | 41.87 | 46.27 | 49.15 | 1.173 | <0.001 | <0.001 | 0.471 |
| Fatty acid (FA) intake, g/d | | | | | | | | | | | | |
| 14:0 | 1.2^CD | 1.0^D | 14.5^A | 9.0^B | 0.1^E | 0.1^E | 1.7^C | 1.1^D | 0.066 | <0.001 | <0.001 | <0.001 |
| 16:0 | 64.2^D | 151.5^B | 96.3^C | 353.3^A | 7.4^G | 19.0^F | 11.2^G | 43.0^E | 1.76 | <0.001 | <0.001 | <0.001 |
| cis-9 16:1 | 0.5^C | 1.2^A | 0.9^B | 0.4^C | 0.06^F | 0.14^D | 0.11^E | 0.05^F | 0.007 | <0.001 | <0.001 | <0.001 |
| 18:0 | 8.3^CD | 20.0^C | 9.4^CD | 307.4^A | 1.0^D | 2.7^D | 1.1^D | 37.2^B | 1.43 | <0.001 | <0.001 | <0.001 |
| cis-9 18:1 | 57.3^B | 311.8^A | 49.5^BC | 57.9^B | 6.8^D | 38.8^C | 5.8^D | 7.4^D | 1.39 | <0.001 | <0.001 | <0.001 |
| cis-11 18:1 | 2.8^B | 8.1^A | 3.3^B | 2.9^B | 0.3^D | 1.0^C | 0.4^D | 0.4^D | 0.041 | <0.001 | <0.001 | <0.001 |
| 18:2n-6 | 145.9^B | 605.3^A | 120.3^B | 128.2^B | 17.2^D | 75.1^C | 14.1^D | 16.3^D | 2.76 | <0.001 | <0.001 | <0.001 |
| 18:3n-3 | 74.4^AB | 56.6^C | 84.0^A | 70.0^B | 8.2^DE | 6.8^E | 9.7^D | 8.5^DE | 0.724 | <0.001 | <0.001 | <0.001 |
| 20:5n-3 | ND[3] | ND | 1.1 | 2.26 | ND | ND | 0.13 | 0.273 | 0.469 | <0.001 | — | — |
| 22:5n-3 | 2.0^B | 1.7^B | 3.0^A | 1.9^B | 0.2^D | 0.2^D | 0.3^C | 0.2^D | 0.021 | <0.001 | <0.001 | <0.001 |
| 22:6n-3 | 0.05^C | 0.5^C | 115.5^A | 0.04^C | 0.01^C | 0.07^C | 13.4^B | 0.01^C | 0.417 | <0.001 | <0.001 | <0.001 |
| Total FA | 400.6^D | 1,206.2^A | 603.4^C | 995.8^B | 46.3^H | 149.9^E | 70.1^G | 122.1^F | 7.26 | <0.001 | <0.001 | <0.001 |
| Yield | | | | | | | | | | | | |
| Milk, kg/d | 27.8 | 25.0 | 26.5 | 27.1 | 3.1 | 3.0 | 2.9 | 3.0 | 0.690 | <0.001 | 0.102 | 0.113 |
| Milk, g/d per kg of BW | 41.3 | 36.6 | 39.4 | 40.2 | 56.9 | 56.2 | 53.7 | 54.3 | 3.11 | 0.002 | 0.246 | 0.231 |
| Fat, g/d | 944^A | 474^C | 703^B | 1,031^A | 106^D | 101^D | 84^D | 107^D | 27.3 | <0.001 | <0.001 | <0.001 |
| Fat, g/d per kg of BW | 1.40^B | 0.69^D | 1.04^C | 1.53^AB | 1.96^A | 1.90^A | 1.55^B | 1.96^A | 0.10 | <0.001 | <0.001 | <0.001 |
| Protein, g/d | 864 | 814 | 819 | 819 | 100 | 101 | 96 | 97 | 16.5 | <0.001 | 0.433 | 0.485 |
| Lactose, g/d | 1,462 | 1,279 | 1,315 | 1,391 | 153 | 157 | 146 | 148 | 36.7 | <0.001 | 0.059 | 0.053 |
| Σ <C16, mmol/d per kg of BW | 2.25^DE | 0.58^F | 1.93^E | 2.19^DE | 4.12^A | 3.00^CD | 3.51^BC | 3.89^AB | 0.20 | <0.001 | <0.001 | 0.004 |
| Σ C16, mmol/d per kg of BW | 1.62^BD | 0.63^F | 1.12^E | 1.95^AC | 2.00^AB | 1.54^CDE | 1.57^CDE | 2.15^A | 0.11 | 0.004 | <0.001 | <0.001 |
| Σ >C16, mmol/d per kg of BW | 1.76^BC | 1.31^D | 1.17^D | 1.98^BC | 2.09^B | 3.03^A | 1.43^CD | 2.14^B | 0.11 | 0.001 | <0.001 | <0.001 |
| Σ 18:0 + cis-9 18:1, mmol/d per kg of BW | 1.17^B | 0.706^C | 0.368^C | 1.43^AB | 1.25^B | 1.77^A | 0.364^C | 1.40^B | 0.070 | 0.010 | <0.001 | <0.001 |
| Concentration, g/100 g | | | | | | | | | | | | |
| Fat | 3.39^BC | 1.85^E | 2.64^D | 3.82^A | 3.47^AB | 3.45^AB | 2.95^CD | 3.62^AB | 0.907 | 0.002 | <0.001 | <0.001 |
| Protein | 3.08^B | 3.29^A | 3.12^AB | 3.03^B | 3.35^AB | 3.37^AB | 3.41^AB | 3.36^AB | 0.946 | 0.086 | 0.024 | 0.043 |
| Lactose | 5.21^A | 5.04^AB | 4.97^B | 5.15^A | 4.98^AB | 5.14^AB | 5.05^AB | 5.01^AB | 0.404 | 0.448 | 0.120 | <0.001 |
| Ratio (g/100 g of FA) | | | | | | | | | | | | |
| trans-11,cis-13 CLA/trans-11 18:1 | 0.0007^AB | 0.0010^A | 0.0003^C | 0.0006^BC | 0.0005^BC | 0.0003^C | 0.0004^BC | 0.0003^C | 0.0001 | <0.001 | 0.002 | <0.001 |
| Energy balance,[4] % | 95^D | 118^AB | 104^CD | 98^D | 105^BCD | 111^ABCD | 114^ABC | 123^A | 2.78 | 0.023 | <0.001 | <0.001 |
| Protein balance,[5] % | 113^BC | 112^BC | 126^AB | 104^C | 126^AB | 105^C | 138^A | 131^AB | 3.42 | 0.031 | <0.001 | <0.001 |

A–F Means within a row not sharing a common superscript differ (P < 0.05) due to species by diet interactions.

[1] Control = basal diet containing no additional oil; COS = basal diet containing corn oil and wheat starch; MAP = basal diet containing marine algae powder; HPO = basal diet containing hydrogenated palm oil.

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

[3] Not detected.

[4] Net energy for lactation balance (MJ/d) calculated according to INRA (2007) and expressed as a percent of estimated requirements.

[5] Protein balance (g of digestible protein in the intestine/d) calculated according to INRA (2007) and expressed as a percent of estimated requirements.

**Table 3.** Messenger RNA relative abundance of genes involved in lipid metabolism in the mammary tissue of cows and goats fed diets supplemented with corn oil and starch or marine algae powder or hydrogenated palm oil (arbitrary units determined as the abundance relative to the arithmetic mean of *RPLP0*, *UXT2*, and *EIF3K* mRNA)[1]

| Pathway and genes | Cows | | | | Goats | | | | SEM | P-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | COS | MAP | HPO | Control | COS | MAP | HPO | | Sp | D | Sp × D |
| Lipid metabolism | | | | | | | | | | | | |
| *ACSL1* | 1.36 | 1.19 | 1.09 | 1.25 | 0.529 | 0.802 | 0.505 | 0.547 | 0.117 | 0.001 | 0.528 | 0.445 |
| *CD36* | 1.52 | 1.56 | 1.33 | 1.40 | 2.19 | 2.09 | 2.45 | 2.25 | 0.155 | 0.002 | 0.991 | 0.593 |
| *FABP3* | 11.85 | 13.79 | 10.54 | 12.36 | 26.61 | 26.22 | 19.97 | 22.40 | 1.66 | <0.001 | 0.252 | 0.708 |
| *FADS3* | 0.0050 | 0.0046 | 0.0050 | 0.0050 | 0.0009 | 0.0001 | $9.22^{-19}$ | $1.79^{-18}$ | 0.001 | <0.001 | 0.940 | 0.959 |
| *FASN* | 0.117 | 0.058 | 0.065 | 0.117 | 0.148 | 0.166 | 0.198 | 0.141 | 0.014 | 0.001 | 0.885 | 0.125 |
| *GPAM* | 0.083 | 0.071 | 0.047 | 0.118 | 0.558 | 0.508 | 0.502 | 0.530 | 0.036 | <0.001 | 0.762 | 0.948 |
| *LPIN1* | 0.061 | 0.026 | 0.026 | 0.078 | 0.201 | 0.166 | 0.147 | 0.178 | 0.014 | <0.001 | 0.154 | 0.810 |
| *LPL* | 0.028[c] | 0.010[c] | 0.012[c] | 0.012[c] | 0.096[ab] | 0.102[ab] | 0.081[b] | 0.121[a] | 0.009 | <0.001 | 0.129 | 0.064 |
| *SCD1* | 1.40 | 1.24 | 0.988 | 1.30 | 1.26 | 1.24 | 1.22 | 1.38 | 0.148 | 0.845 | 0.431 | 0.706 |
| *SCD5* | 0.013 | 0.013 | 0.018 | 0.014 | 0.013 | 0.012 | 0.010 | 0.011 | 0.001 | 0.108 | 0.832 | 0.199 |
| *SLC2A1* | 0.082 | 0.071 | 0.078 | 0.068 | 0.088 | 0.084 | 0.070 | 0.098 | 0.006 | 0.216 | 0.667 | 0.262 |
| Protein metabolism | | | | | | | | | | | | |
| *CSN2* | 126 | 133 | 124 | 215 | 884 | 1,104 | 1,037 | 1,070 | 48.9 | <0.001 | 0.515 | 0.735 |
| *MFGE8* | 0.658 | 0.765 | 0.451 | 0.853 | 1.65 | 1.10 | 1.80 | 1.76 | 0.169 | 0.001 | 0.619 | 0.353 |
| *MTOR* | 0.023 | 0.018 | 0.019 | 0.023 | 0.025 | 0.023 | 0.022 | 0.021 | 0.022 | 0.337 | 0.353 | 0.530 |
| Transcription factor | | | | | | | | | | | | |
| *INSIG1* | 0.165 | 0.078 | 0.097 | 0.213 | 0.703 | 0.491 | 0.445 | 0.658 | 0.056 | <0.001 | 0.040 | 0.645 |
| *LXRA* | 0.030 | 0.024 | 0.031 | 0.037 | 0.031 | 0.020 | 0.015 | 0.020 | 0.026 | 0.024 | 0.178 | 0.130 |
| *PPARA* | 0.026 | 0.015 | 0.019 | 0.023 | 0.013 | 0.010 | 0.013 | 0.010 | 0.001 | <0.001 | 0.038 | 0.111 |
| *PPARG1* | 0.083 | 0.084 | 0.076 | 0.088 | 0.020 | 0.012 | 0.012 | 0.012 | 0.010 | <0.001 | 0.628 | 0.620 |
| *SP1* | 0.046 | 0.027 | 0.038 | 0.046 | 0.041 | 0.033 | 0.040 | 0.037 | 0.002 | 0.611 | 0.010 | 0.248 |
| *SREBF1* | 0.033 | 0.023 | 0.028 | 0.042 | 0.029 | 0.028 | 0.030 | 0.029 | 0.004 | 0.632 | 0.434 | 0.480 |
| Inflammation | | | | | | | | | | | | |
| *TLR4* | 0.048 | 0.037 | 0.043 | 0.050 | 0.052 | 0.052 | 0.046 | 0.055 | 0.003 | 0.081 | 0.299 | 0.641 |

[a–c]Means (n = 12) within a row not sharing a common superscript differ (*P* < 0.10) due to species by diet interactions.

[1]Control = basal diet containing no additional oil; COS = basal diet containing corn oil and wheat starch; MAP = basal diet containing marine algae powder; HPO = basal diet containing hydrogenated palm oil.

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

*LXRA*, and *PPARG1*), and 9 were more abundant in goats (*FASN*, *CD36*, *FABP3*, *LPL*, *GPAM*, *LPIN1*, *CSN2*, *MFGE8*, and *INSIG1*; Table 3).

The dietary treatments had an effect (*P* < 0.05) on the abundance of only 3 mRNA encoding for genes involved in the regulation of lipid metabolism in the mammary gland in both species (Table 3): COS decreased mRNA abundance of *PPARA*, *INSIG1*, and *SP1*, and MAP decreased mRNA abundance of *INSIG1*.

Because of a Sp × D interaction, a tendency (*P* < 0.10) for significance for *LPL* was observed; *LPL*

mRNA tended to be less abundant in goats with MAP and more abundant with HPO compared with CTL, whereas no effect was observed in cows. The dietary treatments had no effect on activities of the 4 enzymes in the mammary tissue collected by biopsy in cows at the end of each experimental period (n = 48; Table 4).

## DISCUSSION

This study is the second part of a direct comparison trial with dairy goats and cows fed various lipids. The

**Table 4.** Enzyme activity (nmol/min per milligram of protein) in the mammary tissue of cows fed diets supplemented with corn oil and starch or marine algae powder or hydrogenated palm oil[1]

| Item | Diet | | | | SEM | P-value[2] |
|---|---|---|---|---|---|---|
| | Control | COS | MAP | HPO | | |
| Fatty acid synthase | 34.68 | 32.60 | 25.26 | 34.80 | 7.814 | 0.733 |
| Malic enzyme | 4.880 | 4.993 | 5.173 | 5.672 | 0.914 | 0.896 |
| Glucose-6-phosphate dehydrogenase | 50.70 | 46.56 | 51.11 | 53.07 | 5.529 | 0.834 |
| Glycerol-3-phosphate dehydrogenase | 324.7 | 297.7 | 336.4 | 331.9 | 55.63 | 0.943 |

[1]Control = basal diet containing no additional oil; COS = basal diet containing corn oil and wheat starch; MAP = basal diet containing marine algae powder; HPO = basal diet containing hydrogenated palm oil.

[2]Probability of significant effects due to experimental diet (D).

first part was dedicated to dairy performances demonstrating strong species specificities in milk fat secretion and fatty acid composition (Fougère et al. 2018), with the main data summarized in Table 2. The present study examined the responses of mammary lipid metabolism.

### Milk Fat Production and Composition

The data on animal performance and milk production and composition are thoroughly reported and discussed in Fougère et al. (2018). The direct comparison of cows and goats performances in response to the COS, MAP, and HPO treatments (with mean milk fat contents of 3.39 vs. 3.47 for the control, 1.85 vs. 3.45 for COS, 2.64 vs. 2.95 for MAP, and 3.82 vs. 3.62 for HPO in cows and goats, respectively; Fougère et al., 2018) confirmed interspecies differences in mammary lipogenesis, which were explored in part in the present study. In cows, COS and MAP treatment induced a decrease in the milk output of short-, medium-, and long-chain FA (<C16, C16, and >C16) expressed as millimoles per day and kilogram of BW (Table 2), which is consistent with characterized MFD (Bauman and Griinari, 2003; Toral et al., 2015). In goats, COS induced a decrease in short- and medium-chain FA that was compensated by an increase in long-chain FA taken up from blood, allowing milk secretion to be maintained, in agreement with other trials in goats receiving diets rich in starch and PUFA but contrary to cows (Chilliard et al., 2007; Toral et al., 2015). However, the MFD observed with MAP in goats was associated with decreases in the output of all FA when expressed per kilogram of BW, although to lesser extent than that in cows (Table 2). In cows fed HPO, only medium-chain FA ($\Sigma$C16) expressed per kilogram of BW increased with milk fat content. These data outlined species specificities in the regulation of mammary lipogenesis response to COS and HPO treatments. Among the mechanisms involved in this regulation and in the differences in responses between species, our previous study (Fougère et al., 2018) showed a dramatic and higher increase in milk trans-10 isomers, in particular trans-10,cis-12 CLA, with COS treatment in cows than in goats. However, most likely other BH intermediates were involved, such as increased in milk trans-7,cis-9 CLA, trans-9,trans-11 CLA, and trans-10,trans-12 CLA, as well as changes in circulating precursors of de novo FA synthesis, such as a decrease in acetate and BHB. Changes linked to MAP-induced MFD: a decrease in 18:0 and cis-9 18:1, and an increase in trans-18:1 in milk were similar in cows and goats. The HPO increase in milk fat content (but not fat yield) observed only in cows was accompanied by an increase in the yield of 16-carbon FA (and

the sum of 18:0 and cis-9 18:1, although not significant) in milk, suggesting that cows had a greater ability to incorporate 16:0 in milk fat than that of goats, which could be due to differences in postabsorptive tissue metabolism. However, to better understand the underlying mechanisms of these responses, a thorough analysis of indicators of lipid metabolism in the rumen, plasma, and mammary gland is a prerequisite, and the aim of the present study was to explore, at first, mammary lipogenesis.

### Mammary Metabolism

*Species Specificities.* A direct comparison of mRNA abundance of the 21 genes studied for cows and goats fed similar diets provided clear evidence of interspecies differences for 14 of the genes involved in milk component synthesis, which suggested differences in mammary metabolism between the ruminant species. First, the mRNA abundance of 5 genes involved in lipid metabolism (ACSL1, FADS3, PPARA, LXRA, and PPARG1; Table 3) was more important in cows than in goats. The FADS3 gene in ruminant mammal genomic sequence databases, as well as the role of the corresponding encoding protein $\Delta$13-desaturase in the endogenous synthesis of trans-11,cis-13 CLA from $\Delta$13-desaturation of vaccenic acid have been demonstrated (Garcia et al., 2017). In accordance with the highest expression of FADS3 in cows compared with that in goats, higher desaturation ratios of trans-11,cis-13 CLA/trans-11 18:1 were observed in cows than those in goats (Table 2). The higher abundance of mRNA of the PPARA, LXRA, and PPARG1 transcription factors in cows than that in goats suggested a higher transcriptional activity of their target genes. However, for PPARG1, among its target genes, FASN, LPIN1, and INSIG1 (Kadegowda et al., 2009) were not higher expressed in cows compared with goats. In addition, mRNA abundance of 9 genes was higher in goats than that in cows (FASN, CD36, FABP3, LPL, GPAM, LPIN1, CSN2, MFGE8, and INSIG1). This result for FASN mRNA abundance is consistent with previous data (Bernard et al., 2017), which suggest a higher de novo synthesis in goats than that in cows in accordance with the higher <C16 sum (in mmol/d per kilogram of BW; Table 2) observed in goats. However, in the absence of the corresponding protein content or activities, a cause and effect relationship between mammary mRNA and milk <C16 FA content is difficult to conclude. Furthermore, the highest mRNA abundance of CD36, LPL, and FABP3 in goats compared with that in cows might be related to the higher milk FA >C16 sum (in mmol/d per kilogram of BW; Table 2) observed in this species, which are products of the

pathways in which these genes are involved, namely long-chain FA uptake and transport by the mammary gland. The mRNA abundance of *CSN2* was higher in goats than that in cows, as previously observed in cows and goats (Bernard et al., 2017).

A higher mRNA abundance of *MFGE8*, a protein of the milk fat globule, observed in goats, together with the smaller average fat globule size (goats; 3.5 µm) in this species than in cows (4.0 µm; Park et al., 2007), could be related to previous results in different goat $\alpha_{S1}$-CN genotypes showing a negative association between *MFGE8* protein and milk fat globule size (Cebo et al., 2012). Moreover, the results of the study demonstrated significant correlations between most of the studied genes. For example, in both species, *LPIN1* and *INSIG1* were significantly and positively associated (r = 0.791, n = 12, $P < 0.001$ and r = 0.724, n = 12, $P < 0.001$ in cows and goats, respectively). These correlations could be related to recent data that report the concomitant regulation of miR-26a/b and their target genes among which are *LPIN1* and *INSIG1* in goat mammary epithelial cells (Wang et al., 2016).

***Responses to COS, MAP, and HPO.*** Although the supplements of corn oil and wheat starch dramatically lowered the milk fat content and yield (g/d) in cows (−45 and −50%, respectively; Fougère et al., 2018) in contrast to goats, 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 previous studies reporting similar MFD with plant oil in bovines (−43% in Piperova et al., 2000; −27% in Peterson et al., 2003) together with decreases in mRNA or the activity of lipogenic enzymes.

As with the COS treatment, MAP induced little or no variation in mammary mRNA or enzyme activities, whereas this treatment lowered milk fat content and yield (g/d) in cows (−22 and −26%, respectively; Fougère et al., 2018) and only milk fat content in goats (−15%). These results are not consistent with previous studies in bovines that reported lowered milk fat content and yield with diets supplemented with fish oil (−34% in Ahnadi et al., 2002) or supplemented with a mixture of plant oil and marine algae (−39% in Angulo et al., 2012), as this was associated with large decreases in lipogenic gene expression in the mammary gland. In most cases, however, these data are consistent with a recent study on dairy cows and goats receiving diets supplemented with either sunflower oil and starch or fish oil (Bernard et al., 2017), which reported decreasing milk fat yield (−31% for both diets in cows or no effect in goats) that was not associated with decreasing mRNA abundance or enzyme activities of lipogenic genes. However, the expression of a few genes

was modified by diets in the present study. Indeed, COS decreased mRNA abundance of *PPARA* by 42% in cows, in contrast to data reported in Bernard et al. (2017) with sunflower oil and starch. Moreover, COS and MAP decreased mRNA abundance of *INSIG1* in both species, by 53 and 41%, respectively, in cows and by 30 and 37%, respectively, in goats. These results are not consistent with previous data (Leroux et al., 2016) that reported no variation in *INSIG1* in cows fed a high forage supplemented with whole intact rapeseeds supplements; however, no variation of milk fat yield was observed in that study. In goats, the decrease of mRNA abundance of *INSIG1* fed COS and MAP is in accordance with the reported decrease of *INSIG1* mRNA in ovines (Carreño et al., 2016) fed diets supplemented with fish oil, which induced a decrease of 22% in milk fat yield. Last, the 41% decrease in the mRNA abundance of *SP1* in cows fed COS is not consistent with the absence of variation for this gene under similar dietary treatment (sunflower oil plus starch supplement in Bernard et al., 2017).

These slight variations in mRNA abundance observed in response to dietary treatments and their differences with previous studies might be partly attributable to methodological differences, including the time of mammary tissue sampling relative to concentrate distribution and milking. In most of the studies in which mammary biopsies are performed, the time of sampling relative to milking and the last meal is not available except for a few studies (Baumgard et al., 2002; Harvatine and Bauman, 2006; Ticiani et al., 2016) in which the biopsy was specified at 1 to 5 h after morning milking and (most likely) feeding. In contrast to those studies, in Bernard et al. (2017) mammary biopsies were obtained before the morning milking and feeding, which was at least 16 h after the evening meal and milking. Under these conditions, the absence of variations in gene expression in response to MFD diets is attributed to post-transcriptional or post-translational regulation for these genes and to short-term regulation of mRNA synthesis by nutrient supply (Chen et al., 2008); in addition, an accumulation of milk in mammary epithelial cells limits expression of genes implicated in milk synthesis (Wall and McFadden, 2010). For these reasons, in the present study mammary biopsies were performed at 5 to 7 h after the morning milking and feeding. However, under these conditions we observed only a few changes in mRNA abundance of lipogenic genes in response to dietary treatments that induced large variation in milk fat composition, at least in cows. As indicated above, among the factors that control the synthesis of milk components are an adequate supply of precursors for mammary lipogenesis and a local regulation when milk accumulates in the udder (Thivierge et

al., 2002). Concerning the adequate supply of precursors, although ruminant compared with nonruminant species present a more constant nutrient delivery to tissues due to the high retention time of feed particles in the rumen, variation in arterial concentrations of the precursors of de novo milk synthesis in sheep throughout the feeding cycle is reported (Rémond et al., 2003), with a maximum observed for acetate and butyrate (precursors of mammary lipogenesis) from 2 to 5 h after the feed distribution (Rémond et al., 2003). Another study in dairy cows reported that the blood and plasma net fluxes of AA precursors of protein synthesis reached their maximum over the first 8 h after milking (Thivierge et al., 2002). Therefore, the effect of changes in nutrient supply over a feeding cycle on mammary gene expression cannot be eliminated when explaining discrepancies among studies that differ in biopsy time relative to feeding. Additionally, the accumulation of milk in the mammary epithelial cells is most likely a major factor that drives gene expression (Wall and McFadden, 2010), which requires further exploration, in particular for lipogenesis pathways. Indeed, in bovines, reduced frequency of milk removal decreases the expression of genes involved in milk synthesis (Littlejohn et al., 2010), suggesting that the regulation of milk synthesis and secretion is controlled mostly through local (intramammary) mechanisms. Most likely, the filling level of the udder via local regulation by udder distension explained, at least in part, the slight variation in gene expression observed in this study (3 of 21 total candidate genes).

Of the 13 common genes studied in the present study and in Bernard et al. (2017)—both using similar mammary sampling procedure and methodology for the measurement of mRNA abundance but differing in tissue sampling time relative to milking and feeding—1 gene responded to COS treatment in the present study in both species (*INSIG1*), whereas no variation was observed under similar dietary treatment in Bernard et al. (2017). Comparison of the data for similar genes between these studies suggests that mammary sampling at 5 to 7 h after the morning milking and feeding, compared with sampling at 16 h after the evening milking and feeding, altered the expression of few genes response to dietary treatment. These differences might be related to the mechanisms mentioned above.

In cows, but not in goats, HPO led to an increase in milk fat content of 13% (and not yield) and milk 16:0 + *cis*-9 16:1 and 18:0 + *cis*-9 18:1 concentrations of 11%, on average (Fougère et al., 2018), without affecting mammary mRNA and enzyme activities. This suggested that other mechanisms were involved, such as differences in postabsorptive tissue metabolism between these 2 species. However, the lack of data on circulating FA in the plasma of these 2 species under similar dietary conditions does not allow confirmation of this hypothesis.

Pearson correlations performed between the abundance of the 21 mRNA and the yield (g/d) of milk individual FA revealed 10 significant correlations with r >0.40 or r <−0.40 in goats and 25 significant correlations in cows (results not presented). Among those in goats, 2 positive correlations were observed between *SCD5* and the milk desaturation ratios of *cis*-9 14:1/14:0 and *cis*-9,*trans*-11 18:2/*trans*-11 18:1, and 3 positive correlations were observed between *LPL* and *cis*-9,*trans*-11 CLA, *trans*-8,*cis*-10 CLA, and 20:3n-6; however, these associations require further investigation. Notably, specifically in cows, the transcription factor *SP1* was correlated with the yield (g/d) of 14 individual FA (6:0, 8:0, *cis*-9 10:1, *iso*-13:0, *iso*-14:0, *iso*-15:0, *iso*-16:0, 14:0, *trans*-9 14:1, *anteiso*-15:0, *anteiso*-17:0, *cis*-7 16:1, 18:3n-3, 24:0) or FA sum (C4-C14, <C16) or desaturation ratios, which require further investigation because of the role of this transcription factor in a wide range of cellular processes in mammalian cells (Chen et al., 2018).

In cows and goats, COS and MAP decreased <C16 FA expressed in millimoles per day per kilogram of BW (although the decrease was compensated in goats by an increase in >C16) and *INSIG1* mRNA abundance, consistent with previous data with ovines fed diets supplemented with lipid-encapsulated CLA (mixture of *cis*-9,*trans*-11 and *trans*-10,*cis*-12; Hussein et al., 2013). Collectively, these data suggest a role for *INSIG1* in the regulation of mammary de novo lipogenesis in ruminants fed diets supplemented with PUFA-rich lipids. However, this putative role for *INSIG1* was not related to mRNA abundance of de novo lipogenic genes in the present study summarized herein.

In our study, the transcriptional responses of the 21 genes to dietary treatments underlined changes in 3 transcription factors, suggesting differences of dynamic response between transcription factors and their target genes, as shown on plant and eukaryote dynamic genes regulatory network (Li et al., 2015). The absence of responses to dietary treatments in terms of the activities of lipogenic enzymes measured 5 to 7 h after morning milking and feeding in cows, despite MFD, is in accordance with previous data for cows fed sunflower oil plus starch or fish oil lipid supplements 16 h after evening milking and feeding (Bernard et al., 2017); this emphasizes that the variations in milk fat secretion observed with these diets were not due to variations in these activities, and therefore other mechanisms are involved. It cannot be ruled out that the absence of response in terms of enzyme activities could also be partly explained by the time of sampling relative to

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milking and feeding. Moreover, these potential enzyme activities (as measured in vitro under optimal conditions) most likely do not represent the in vivo activity.

## CONCLUSIONS

This study examined the mRNA abundance of mammary lipogenic genes to determine whether they were related to the differences in responses of milk fat content, milk fat yield, and composition observed with various lipid supplements on dairy cows and goats. We observed no variation in the mammary mRNA or enzyme activities due to the diets, although COS dramatically lowered the milk fat content in cows (−45%), as did MAP in both species but to a lesser extent (−22% in cows and −15% in goats), whereas HPO increased milk fat content in cows (+13%); these effects were less significant for fat yields, except for COS and MAP (−50 and −26% respectively) in cows. Only the mRNA abundance of 3 transcription factors, *PPARA*, *SP1*, and *INSIG1*, were affected by COS and MAP treatments. This result could be partly explained by the time of mammary tissue sampling relative to feed distribution and milking due to changes in nutrient supply over a feeding cycle and local regulation linked to milk accumulation in mammary epithelial cells. Additionally, these genes might be regulated at other levels, such as post-transcriptional or post-translational. Moreover, differences in rumen and postabsorptive tissue lipid metabolism are probably involved in the observed differences of milk fat synthesis due to diets between species and require further investigation. Major differences in the abundance of mRNA encoding for genes involved in mammary lipid metabolism were observed between cows and goats, suggesting strong species specificities in the lipogenic pathways or their regulation.

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