Feeding of palm oil fatty acids or rapeseed oil throughout lactation: Effects on mammary gene expression and milk production in Norwegian dairy goats

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ABSTRACT

Lipid added as rapeseed or palm oil to the diet of dairy goats over 8 mo of one lactation alters fat secretion and milk fatty acid (FA) and protein composition. In this study, we examined the contribution of mammary gene expression to these changes and included 30 multiparous goats of Norwegian dairy goat breed for a 230-d experimental period, with indoor feeding from 1 to 120 d in milk (DIM), mountain grazing from 120 to 200 DIM, and indoor feeding from 200 to 230 DIM. After an initial period (1–60 DIM) when the control diet was given to all goats, the animals were subdivided into 3 groups of 10 goats. Treatments (60–230 DIM) were basal concentrate (control) alone or supplemented with either 8% (by weight) hydrogenated palm oil enriched with palmitic acid (POFA) or 8% (by weight) rapeseed oil (RSO). Milk was sampled individually from all animals throughout lactation, at 60, 120, 190, and 230 DIM for milk yield and composition. On d 60, 120, 190, and 230, mammary tissue was collected by biopsy to measure mRNA abundance of 19 key genes. None of the 19 genes involved in milk protein, apoptosis, lipid metabolism, transcription factors, and protein of the milk fat globule membrane, as measured by mRNA abundance, were affected by the lipid supplements, although POFA increased milk fat content, and POFA and RSO affect milk FA composition. Over the experimental period (120–230 DIM), the mRNA abundance of 13 of the 19 studied genes was affected by lactation stage. For some genes, expression either gradually increased from 120 to 230 DIM (CSN2, CASP8, CD36, GLUT4) or increased from 120 to 200 and then remained stable (XDH), or decreased (CSN3, G6PD, SREBF1, PPARG1) or increased only at 230 DIM (SCD1, SCD5, ELF3). For a second group of genes (CSN1, LALBA, FABP3, FASN, LPL, MFGE8), expression was stable over the lactation period. Our results suggest that factors other than gene expression, such as substrate availability or posttranscriptional regulation of these genes, could play an important role in the milk fat and FA responses to dietary fat composition in the goat. In conclusion, mammary gene expression in goats was more regulated by stage of lactation than by the dietary treatments applied.

Key words: goat, lipid supplement, mammary gene expression, lactation period, milk fatty acids

INTRODUCTION

The yields and composition of milk fat and protein are primary determinants of the production efficiency of dairy animals and the nutritional, sensory, and technological qualities for consumers and dairy producers. Among the factors determining milk composition, nutrition dominates (Bernard et al., 2008) but stage of lactation (Vijayakumar et al., 2017) also plays a large role. Nutrition has a particularly strong effect on the composition of milk lipids, whereas proteins and lactose are only marginally affected by this factor (Chilliard et al., 2007), except during times of feed restriction.

To this end, addition of fat supplements such as palm oil has been largely used to increase dietary energy density (Onetti and Grummer, 2004). The use of palm oil is currently under severe criticism from an environmental sustainability point of view (Wilcove et al., 1997; Shingfield et al., 2008). Plant oils such as rapeseed oil are rich in MUFA and PUFA and can be used to change milk fat composition by reducing the fatty acids (FA) synthesized de novo (C10:0–C16:0) and increasing C18:0, C18:1 cis-9, and n-3 PUFA contents without major changes in trans C18:1 and CLA in cows and goats (Chilliard et al., 2003; Bernard et al., 2009c). Such changes are considered beneficial from the consumer’s point of view.

Forages, both fresh and conserved, constitute a major part of the diet of dairy goats in most farming systems. During summer, goats graze forest or...
mountain pastures. Generally, such pastures have rich variety of plants, including grasses and sedges, bushes, and a variety of herbs providing significant amounts of dietary PUFA. Grass-based diets can thus improve the nutritional quality of produced milk by shifting the FA composition toward less SFA and more PUFA, especially n-3 (omega-3) FA (Dewhurst et al., 2006).

Milk composition and, in particular, milk lipids are affected by stage of lactation (Inglingstad et al., 2016); body lipid stores vary greatly during the lactation cycle (Eknæs et al., 2017). In early lactation, when feed intake is insufficient to meet the goat’s need for nutrients, milk fat contains a large amount of long-chain FA originating from mobilized body fat. When adequate feed resources are available in late lactation, goats usually rebuild their fat reserves in preparation for the next lactation, and less fat is secreted into the milk (Eknæs et al., 2017).

Understanding the mechanism of milk synthesis is relevant because milk components affect manufacturing properties and organoleptic qualities of milk and dairy products. The synthesis of milk components is under the control of a large number of genes whose regulation of expression by nutritional and physiological factors may explain, at least in part, the variation in milk components. Thereby, the objective of the present study was to examine mammary metabolism by measuring mammary expression of genes related to lipogenesis and protein synthesis in goats receiving supplements of palm oil–derived fatty acids (rich in SFA) or rapeseed oil (rich in PUFA) over a full lactation period. The effects of these plant oils on dairy performance, composition, and mammary metabolism have never been studied in goats over a lactation period. Our second objective was to explore the relationships between gene expression data and milk yield and milk composition parameters. This study is part of a larger trial performed on the same goats studying the expression of genes involved in milk protein and fat synthesis.

**MATERIALS AND METHODS**

**Experimental Design**

The experiment was carried out at the Norwegian University of Life Sciences, Department of Animal and Aquaculture Sciences (Ås, Norway) in agreement with the laws and regulations controlling experiments on live animals in Norway and under the supervision of the Norwegian Animal Research Authority.

The experiment included 30 Norwegian dairy goats in second to fourth lactation, kidding from February 3 to March 7 (average kidding date: February 17 ± 8 d) and with average BW 2 d after kidding of 54.4 ± 6.7 kg. The experiment lasted for 230 d and consisted of 3 periods (P): indoor feeding (P1) from 1 to 120 DIM, mountain grazing (P2) from 120 to 200 DIM, and indoor feeding (P3) from 200 to 230 DIM. All goats were fed a basal concentrate without added fat (control; CON) until 60 DIM (preparatory period). Thereafter, the goats were allocated to 3 groups, each of 10 goats, based on age, date of kidding, BW, milk yield, and genotype [homozygous (E12–00) or heterozygous (E12–01) for the deletion in exon 12 at the αS1-CN locus]. Each group consisted of 7 goats heterozygous (E12–01) for the deletion in exon 12 and 3 goats homozygous (E12–00) for this deletion.

**Animal Management and Experimental Diets**

During the spring and autumn indoor feeding periods (P1 and P3, respectively), goats were kept in individual stalls at the experimental farm at Ås (59°39′N; 10°46′E), 90 m above sea level (m.a.s.l.), and fed grass silage consisting of timothy (Phleum pratense L.), meadow fescue (Festuca pratensis Huds.), and red clover (Trifolium pratense L.) according to appetite (targeting 10% refusals). Mountain pastures were located in Follo (62°19′N; 10°1′E), above the treeline (900 to 1,000 m.a.s.l.), and consisted of marsh areas with sedges (mainly Carex nigra and Carex rostrata) and drier areas with grasses (mainly Deschampsia cespitosa and Deschampsia flexuosa), willow thickets (Salix spp.), birch (Betula spp.), and a variety of herbs. The goats grazed freely day and night.

Concentrates were provided at 0.9 kg/d during P1 and at 0.7 kg/d during P2 and P3. The daily concentrate ration was fed individually to the goats in 3 equal meals during P1 and P3 and in 2 equal meals during P2. The goats were milked in a milking stable at 0630 and 1600 h. Further details on feeding management and...
The 3 experimental concentrates were as follows: (1) CON concentrate, (2) CON concentrate containing 8% (by weight) hydrogenated palm oil fatty acids enriched with palmitic acid (AkoFeed Gigant 60, AAK Sweden AB; POFA), and (3) CON concentrate containing 8% (by weight) rapeseed oil (AAK Sweden AB; RSO). The experimental concentrate mixtures (Table 1) were produced by the Centre for Feed Technology at the Norwegian University of Life Sciences (Ås, Norway).

**Measurement and Sampling**

Feed intake, chemical composition of experimental diets, and milk yields were determined for each lactation period according to sampling protocols and analytical procedures outlined in Eknæs et al. (2017). Milk was sampled from all animals 4 times throughout the lactation period, at 60, 120, 190, and 230 DIM. Milk samples for analysis were a proportional mix of evening and morning milk, pooled in the ratio 1.5:1, based on estimated average ratios between morning and evening milk yields.

Approximately 30 mg of mammary tissue was collected from both glands (for the 5 periods; at 30, 60, 120, 190, and 230 DIM). Biopsies were rinsed in sterile saline solution and inspected visually to verify the homogeneity of the secretory tissue sample before being snap-frozen in liquid N2 and stored at −80°C until RNA.
extractions. Biopsy collections resulted in slight bleeding on some occasions; in these cases, milk appeared normal after 1 to 3 milkings. During the postsampling period, extreme care was taken during milking to remove any blood clots lodged in the glands. No intramammary infections or loss of milk production was encountered after 1 to 3 milkings. During the postsampling period, milk appeared normal on some occasions; in these cases, milk appeared normal

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

Total RNA was prepared through the homogenization of approximately 20 mg of biopsy tissue in 0.35 mL of TRizol reagent (Invitrogen Life Technologies), followed by isolation using the Pure Link RNA mini kit isolation system (Invitrogen), according to the manufacturer’s instructions. Potential contaminating genomic DNA was removed through a DNase treatment step (RNase-Free DNase Set #79254, Qiagen). The RNA content was determined by measuring absorbance at 260, 280, and 320 nm using a NanoDrop (ND-1000 spectrophotometer; NanoDrop, Labtech). RNA integrity was determined using a 2100 Bioanalyzer (Agilent Technologies) and averaged 7.6 (SD 0.79).

Reverse transcription was performed from 2 µg of purified total RNA isolated from the mammary samples using the High-Capacity RNA-to-cDNA kit containing dNTPs, random octamers, and oligo dT-16 (Applied Biosystems) 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 real-time PCR: FASN and G6PD involved in de novo FA synthesis; LPL, CD36, FABP3, and GLUT4 involved in FA uptake (the 3 former) and glucose uptake (the latter), respectively; SCD1 and SCD5, involved in FA desaturation; GPAM, involved in triglyceride synthesis; LALBA, CSN1S1, CSN2, and CSN3, which encode 4 major proteins in the milk; XDH and MFGE8, which encode the 2 major proteins in the milk fat globule membrane; CASP8 and ELF3, which are involved in the signaling pathways of apoptosis and cells differentiation, respectively; and transcription factors SREBF1 and PPARG1, which are involved in the regulation of lipogenic gene expression.

To account for variation in RNA integrity, RNA quantification, and cDNA synthesis, mRNA abundance was normalized using the geometric mean of 3 housekeeping genes (MRPL39, UXT, EIF3K), which were identified as suitable internal controls for caprine mammary tissues among several tested (Bonnet et al., 2013), and their stability was verified using GeNorm software (https://genorm.cmgg.be/). The mRNA abundance was quantified in duplicate via quantitative real-time PCR using the StepOnePlus real-time PCR system (Applied Biosystems) and SYBR Green dye (TF Power SYBRGreen PCRMasterMix) or a fluorescent TaqMan probe (TF TaqManFast Universal PCR Master Mix), according to the manufacturer’s instructions (Applied Biosystems) and with specific primers and probes (Supplemental Table S1; https://data.mendeley.com/datasets/s8r4pzk69/1; Eknæs and Bernard, 2022). 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 2-step cycle repeated 40 times: denaturing for 15 s at 94°C and annealing and extension for 45 s at 58 or 60°C (depending on 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 45 s at 60°C.

The efficiency of PCR was 88.4% (SD 6.24) for the 19 target genes and 91.4% (SD 0.83) 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.

**Statistical Analysis**

Analysis of variance was performed using the MIXED procedure (Littell et al., 1998) of SAS (2003; SAS Institute Inc.). All measurements were repeated 4 times for each animal, and appeared correlated. Consequently, these correlations were taken into account in the statistical model. Covariance structure of the repeated measurements was chosen by comparing potential structures using Akaike’s and Schwarz’s Bayesian information criterion (Wolfinger, 1996), and a spatial power covariance structure proved useful for all data. The value at 60 DIM was used as a covariate. Gene expression data on 3 goats, 1 for each experimental treatment, gave extremely low values probably related to the technique. These goats were excluded, resulting in a balanced data set with 9 goats per treatment. An ANOVA for repeated measurements was performed according to the following model:

\[
Y_{ijkl} = \mu + A_i + B_j + A \times B_{(ij)} + C_k + A \times C_{(ik)} + \varepsilon_{ijkl}
\]

where \(Y_{ijkl}\) is the dependent variable; \(\mu\) is the intercept; \(A_i\) is the fixed effect of concentrate type, \(i = 1, 2, 3\) (CON, POFA, RSO); \(B_j\) is the fixed effect of DIM, \(j = 1, 2, \ldots, 4\) (DIM 90, 120, 190, 230); \(A \times B_{(ij)}\) is the
interaction between concentrate type \(i\) and DIM \(j\); \(C_k\) is the fixed effect of genotype at the CSN1S1 locus, \(k = 1, 2\) (E12–00, E12–01); \(A \times C_{(ik)}\) is the interaction between concentrate type \(i\) and genotype \(k\); \(pEX\) is the pre-experimental value of the variable (as covariate); \(d(A,C_{ik})\) is random effect of goat within concentrate type \(i\) and genotype \(k\); and \(\varepsilon_{ijlk}\) represents the experimental error.

Differences between means were tested based on least square differences using the default pairwise \(t\)-test in the PDIF option in the lsmeans statement. Pearson correlation coefficients were generated for associations among mammary mRNA abundances and milk yield, milk composition, and LPL activity. Differences were considered statistically significant when \(P < 0.05\), and trends were apparent when \(0.05 < P < 0.10\).

**RESULTS**

**Diet Composition and Intake of FA**

The formulation of experimental concentrates and the chemical composition and FA profile of concentrate and silage are reported in Table 1 and (in more detail) in Eknæs et al. (2017). The concentrate mixtures were isonitrogenous, and the estimated energy contents of POFA and RSO were higher than that of CON. The inclusion of oil resulted in more crude fat in the POFA and RSO treatments than in CON (Table 1). By design, the inclusion of hydrogenated palm oil enriched with palmitic acid resulted in increased intakes of C16:0 and C18:0 in the POFA treatment (Table 2), whereas the addition of rapeseed oil increased the intake of C18:1 cis-9 and C18:3n-3 and, to a lesser extent, C18:2n-6 in the RSO treatment, with C18:1 cis-9 as the primary FA (Table 2).

**Animal Performance**

The effects of treatments on DMI, animal performance, and milk composition are reported in Table 3, and in more detail in Eknæs et al. (2017). Daily DMI was depressed in the POFA group during indoor feeding periods in the spring (120 DIM) and autumn (230 DIM). Energy balance was negative in the early lactating period (60 DIM) and then positive between 120 and 230 DIM for all dietary treatments (Table 3). For all treatments, milk yield decreased throughout lactation from a mean of 3.1 kg/d at 60 DIM to 1.9 kg/d at 230 DIM. Milk fat yield decreased over the lactation period and was higher \((P = 0.01)\) in goats fed POFA at 120 DIM compared with those fed CON. At 190 DIM, milk fat yield was higher \((P < 0.01)\) for both POFA and RSO compared with CON. Milk protein and lactose yields gradually decreased from 60 to 230 DIM (Table 3).

Regardless of the treatment, milk short- and medium-chain FA yields decreased from 60 to 190 DIM (Table 4). The yields of long-chain FA and MUFA decreased from 60 to 120 DIM, remained stable at 190 DIM (mountain grazing), and then decreased at 230 DIM (Table 4). Compared with CON treatment, supplementing POFA led to increased yields of C16 FA and long-chain FA, whereas supplementing RSO induced a large increase in long-chain FA, MUFA, and PUFA (Table 4).

**Mammary Lipid Metabolism**

The mRNA relative abundances of genes involved in de novo FA synthesis, FA and glucose uptake, FA desaturation, triglyceride synthesis, milk protein, protein of the milk fat globule membrane, apoptosis, cell differentiation, and transcription factors were not affected by lipid supplementation (Table 5).

Over the experimental period (120 to 230 DIM), the mRNA relative abundance of studied genes was affected by lactation stage, except for that of CSN1, LALBA, FABP3, FASN, GPAM, LPL, and MFGES, which remained stable over the lactation (Table 5). For the affected 12 genes, their expression either gradually increased from 120 to 230 DIM (CSN2, CASP8, GLUT4), increased from 120 to 200 and then remained stable (XDH), decreased (CSN3, G6PD, SREBF1, PPARG1), or increased only at 230 DIM (CD36, SCD1, SCD5, ELF3) (Table 5).

**DISCUSSION**

This study is the fourth part of a feeding trial with Norwegian dairy goats fed supplements of rapeseed or palm oils over 8 mo of a lactation in which dairy performance, lipid stores, and energy balance (Eknæs et al., 2017); milk FA composition, lipolysis and LPL activity, free fatty acids, and milk fat globule size (Inglingstad et al., 2017); and milk protein and cheesemaking properties (Inglingstad et al., 2016) were previously reported. We observed changes in milk fat yield and FA and protein composition in response to lipid supplements and lactation stage. To better understand the underlying mechanisms of these responses to treatments and lactation stage, a comprehensive analysis of indicators of lipid and protein metabolism in the digestive and postabsorptive tissues including the mammary gland is necessary. Therefore, in the present study, we examined the responses of mammary metabolism by studying the expression of 19 genes involved in central pathways for synthesis of milk components to investigate the relationships between gene expression data and milk...
production parameters, and to study their changes over 8 mo of lactation.

**Responses to Lipid Supplements**

The POFA diet increased milk fat yield and yields of C16 FA (+36%) and long-chain FA (+25%), mainly due to increases in C16:0 and C18:0, respectively (Inglingstad et al., 2017) but had no or little effect on short- and medium-chain FA yields compared with CON and RSO treatments (Eknæs et al., 2017; Inglingstad et al., 2017). However, the observed changes were not accompanied by variations in mammary mRNA abundance of lipogenic genes (Table 5). Conversely, other studies reported an increase in the transcription factor PPARG1 in response to C16:0 supply to bovine mammary cells in vitro, which increased triacylglycerol synthesis and upregulated the FASN target gene (Kadegowda et al., 2009). In accordance with our results, a unique in vivo comparative study in dairy cows and goats fed diets supplemented with hydrogenated palm oil (Fougère et al., 2018) reported specific increases in milk fat content.

### Table 2. Intake of fatty acids (g/d) in goats during the preparatory period (means) and in each experimental group (ismeans) at different stages of lactation

| Fatty acid | Treatment (T) | Experimental period² (DIM) | P-value | SEM³ |
|------------|--------------|----------------------------|---------|------|
|            | P1 60        | P1 120                     | P2 190  | P3 230 |
| C14:0      | CON 0.34     | 0.36b,y                    | 0.41b,x | 0.022 |
|            | POFA 0.34    | 0.85a,x                    | 0.78b,x | <0.01 |
|            | RSO 0.35     | 0.43y                      | 0.38b   | <0.01 |
|            | SEM 0.020    |                            |         | <0.01 |
| C16:0      | CON 8.97     | 9.36c,y                    | 10.18c,x| 0.500 |
|            | POFA 9.12    | 50.20a,x                   | 41.73b  | <0.01 |
|            | RSO 9.32     | 13.23c                     | 12.73b  | <0.01 |
|            | SEM 0.446    |                            |         | <0.01 |
| C16:1 cis-9 | CON 0.72    | 0.77c,y                    | 0.92b,x | 0.056 |
|            | POFA 0.74    | 0.58b,x                    | 0.75b   | <0.01 |
|            | RSO 0.76     | 0.92c                      | 0.99a   | <0.01 |
|            | SEM 0.050    |                            |         | <0.01 |
| C18:0      | CON 0.97     | 1.01c,y                    | 1.11b,x | 0.056 |
|            | POFA 0.99    | 23.02a,x                   | 18.20b  | <0.01 |
|            | RSO 1.01     | 2.76b,x                    | 2.41b   | <0.01 |
|            | SEM 0.050    |                            |         | <0.01 |
| C18:1 cis-9 | CON 4.12    | 4.21c,x                    | 3.99b   | 0.122 |
|            | POFA 4.15    | 4.99b,x                    | 4.54b   | <0.01 |
|            | RSO 4.20     | 4.45b,x                    | 35.06b  | <0.01 |
| C18:1 cis-11 | CON 0.67   | 0.68c                      | 0.66c   | 0.022 |
|            | POFA 0.67    | 0.85b,x                    | 0.78b   | <0.01 |
|            | RSO 0.68     | 2.99a,x                    | 2.43b   | <0.01 |
|            | SEM 0.020    |                            |         | <0.01 |
| C18:2n-6   | CON 13.27    | 13.71b                     | 13.95b  | 0.566 |
|            | POFA 13.43   | 12.08b                     | 12.44b  | <0.01 |
|            | RSO 13.66    | 27.00b                     | 23.74b  | <0.01 |
|            | SEM 0.506    |                            |         | <0.01 |
| C18:3n-3   | CON 18.79    | 20.00b                     | 24.54a  | 1.554 |
|            | POFA 19.23   | 14.93b                     | 19.98b  | <0.01 |
|            | RSO 19.86    | 25.08b                     | 27.16a  | <0.01 |
|            | SEM 1.389    |                            |         | 0.16  |
| C20:0      | CON 0.38     | 0.41b,y                    | 0.51b   | 0.033 |
|            | POFA 0.39    | 0.54b,x                    | 0.60b   | <0.01 |
|            | RSO 0.41     | 0.97a,x                    | 0.92b   | <0.01 |
|            | SEM 0.030    |                            |         | <0.01 |

a–cMeans within a column with different superscripts differ (P < 0.05).

x,yMeans within a row with different superscripts differ (P < 0.05).

1CON (control) = basal concentrate containing no additional fat; POFA = basal concentrate supplemented with hydrogenated palm oil enriched with palmitic acid (AkoFeed Gigant 60, AAK Sweden AB); RSO = basal concentrate supplemented with rapeseed oil (AAK Sweden AB). All goats were fed the CON concentrate from 1 to 60 DIM.

2P1 = indoor feeding; P2 = mountain pasture; P3 = indoor feeding.

3Standard error of mean for the experimental period.

4Forage and fatty acids intakes were not measured on mountain pasture.

5Standard error of means in this column refers to the preparatory period (1–60 DIM).
(+13%) and C16:0 + C16:1 cis-9 and C18:0 + C18:1 cis-9 contents in cows but not in goats. These effects were not accompanied by any changes in mammary mRNA abundance of lipogenic genes, including PPARG1, in either cows or goats. Similarly, a recent study in cows supplemented with free palmitic acid or rapeseed oil compared with a control diet reported increased milk fat content only with palm supplements, which was accompanied by a tendency for higher mRNA abundance of PPARG1 in mammary tissue with palm supplements compared with rapeseed (Bernard et al., 2021). The RSO treatment in the present study had no or little effect on short- and medium-chain FA yields, whereas it induced large increases in long-chain FA content (+65%), mainly due to increased C18:1 cis-9 and C18:2 FA (Inglingstad et al., 2017). The higher content of long-chain FA and UFA was not accompanied by any changes in mRNA abundance of 19 genes in mammary tissue, including those involved in the uptake (LPL, CD36), transport (FABP3), and desaturation (SCD1, SCD5) of FA (Table 5). Our results are in line with previous data in goats fed rapeseed that reported no variation in milk fat yield or mRNA abundance of lipogenic genes (Ollier et al., 2009).

In the RSO group of the present study, the lower content of free FA and off-flavors in milk observed in mid lactation and resulting from milk lipolysis, which is mainly due to the action of the lipase LPL on triacylglycerol (Inglingstad et al., 2017), cannot be explained by higher mRNA abundance of LPL. This result is in accordance with the lack of variation in LPL expression reported in lipid-supplemented goats (Bernard et al., 2009a,b). Otherwise, the activity of LPL may be regulated through mechanisms that operate at the posttranslational level involving a number of extracellular proteins (activator or inhibitor) (Sundheim and Bengtsson-Olivecrona, 1987; Chilliard et al., 2003, 2014).

The specific higher abundance of mammary ELF3 mRNA, which encodes a protein involved in cell differ-
entiation and that is associated with mammary gland development and involution, at 230 DIM for goats on the RSO and POFA treatments could be related to the effect of lipids in increasing mammary cell apoptosis, as observed in human breast cancer (Saggar et al., 2010; Escrich et al., 2019).

The lack of changes or slight variations in mRNA abundance observed in response to dietary treatment are in accordance with other data in goats fed diets supplemented with hydrogenated palm oil (Fougère and Bernard, 2019) and rapeseed oil (Ollier et al., 2009). These results suggest that factors other than gene expression, such as substrate availability for mammary metabolism, could play an important role in the milk fat and FA responses to changes in diet composition in the goat. Moreover, as previously mentioned, these genes might be regulated at other levels, such as the posttranscriptional or posttranslational level. Finally, we cannot exclude an effect of the methodology used, including the time of mammary tissue sampling relative to feed distribution and milking.

### Responses to Lactation Stage

Regardless of diet, mean changes in milk yield (−33%), milk fat and protein (+2 and +11%, respectively), milk fat and protein yields (−32 and −27%, respectively), and in FA yields over the experimental period (Tables 3 and 4; Eknæs et al., 2017) were accompanied by different patterns of mammary mRNA abun-

### Table 4. Milk fatty acid yield (g/d) in goats during the preparatory period (means) and in each experimental group (lsmeans) at different stages of lactation

| Fatty acid | Treatment (T) | Experimental period (DIM) | P-value |
|------------|---------------|---------------------------|---------|
|            |               | P1 60                     | P1 120  | P2 190 | P3 230 | SEM | T   | DIM | T × DIM |
| SCFA CON   | 20.51         | 10.99*                    | 8.03*   | 8.76*   | 0.770 | 0.45 | 0.01 | 0.21 |
| POFA       | 21.67         | 9.65                      | 9.14    | 9.10    |       |      |      |      |
| RSO        | 24.32         | 10.68                     | 9.91    | 10.24   |       |      |      |      |
| SEM        | 1.251         |                          |         |         |       |      |      |      |
| MCFA CON   | 21.86         | 13.10−x                   | 8.90x   | 11.38x  | 0.681 | 0.40 | <0.01 | 0.02 |
| POFA       | 23.49         | 10.46−x                   | 9.84    | 10.63   |       |      |      |      |
| RSO        | 26.30         | 11.34−x,−y                | 10.16x  | 11.89x  |       |      |      |      |
| SEM        | 0.970         |                          |         |         |       |      |      |      |
| C16 FA CON | 39.99         | 31.04−x                   | 22.99y  | 23.74y  | 1.595 | <0.01 | <0.01 | <0.01 |
| POFA       | 39.26         | 27.55−x                   | 39.17x  | 30.05x  |       |      |      |      |
| RSO        | 43.91         | 22.45−x                   | 23.32b  | 20.21b  |       |      |      |      |
| SEM        | 2.139         |                          |         |         |       |      |      |      |
| LCFA CON   | 49.93         | 32.41−x                   | 37.45x  | 15.28xy  | 2.106 | <0.01 | <0.01 | 0.03 |
| POFA       | 48.08         | 41.44−x                   | 45.35x  | 20.97y  |       |      |      |      |
| RSO        | 56.53         | 57.54−x                   | 56.97x  | 28.09y  |       |      |      |      |
| SEM        | 4.439         |                          |         |         |       |      |      |      |
| MUFA CON   | 32.36         | 21.05−x                   | 20.24x  | 10.86y  | 1.286 | <0.01 | <0.01 | 0.19 |
| POFA       | 30.94         | 27.37−x                   | 25.60b  | 15.25y  |       |      |      |      |
| RSO        | 35.83         | 34.68−x                   | 30.54x  | 18.82x  |       |      |      |      |
| SEM        | 3.090         |                          |         |         |       |      |      |      |
| PUFA CON   | 3.69          | 2.65−y                    | 3.56y   | 1.32x   | 0.164 | <0.01 | <0.01 | 0.18 |
| POFA       | 3.69          | 2.32−y                    | 3.61y   | 1.23y   |       |      |      |      |
| RSO        | 4.29          | 3.38−y                    | 4.03y   | 1.65y   |       |      |      |      |
| SEM        | 0.261         |                          |         |         |       |      |      |      |

**a**–**c**Means within a column with different superscripts differ (P < 0.05).

**x**–**z**Means within a row with different superscripts differ (P < 0.05).

1SCFA = short chain fatty acids; C4:0 + C6:0 + C8:0 + C10:0 + C11:0; MCFA = medium-chain fatty acids: C12:0 + C14:0 C14:1 cis-9; C16 FA: C16:0 + C16:1 trans-9 + C16:1 cis-9; LCFA = long-chain fatty acids: C17:0 iso + C17:0 anteiso + C17:0 + C17:1 cis-9 + C18:0 + C18:1 trans-9 + C18:1 trans-11 + C18:1 cis-9 + C18:1 cis-11 + C18:2 cis-9, trans-11 + C18:2 cis-9, trans-11 + C20:0 + C20:1 cis-9,12,15 + C20:1 cis-9, trans-9 + C22:0 + C20:4 cis-5,8,11,14; MUFA: C14:1 cis-9 + C16:1 trans-9 + C16:1 cis-9 + C17:1 cis-9 + C18:1 trans-9 + C18:1 trans-11 + C18:1 cis-9 + C18:1 cis-11 + C20:1 cis-11; PUFA: C18:2 trans-9,12 + C18:2 cis-9,12 + C18:3 cis-9,12,15 + C18:2 cis-9,trans-11 + C20:1 cis-5,8,11,14.

2CON (control) = basal concentrate containing no additional fat; POFA = basal concentrate supplemented with hydrogenated palm oil enriched with palmitic acid (AkoFeed Gigant 60, AAK Sweden AB); RSO = basal concentrate supplemented with rapeseed oil (AAK Sweden AB). All goats were fed the CON concentrate from 1 to 60 DIM.

3P1 = indoor feeding; P2 = mountain pasture; P3 = indoor feeding.

4Standard error of mean for the experimental period.

5Standard error of means in this column refers to the preparatory period (1–60 DIM).
Table 5. mRNA abundance of genes involved in different pathways of protein and lipid metabolism in the mammary tissue (arbitrary units determined as the abundance relative to the geometric mean of MRLP39, UXT2, and EIF3K mRNA) of goats

| Pathway and genes        | Treatment | Experimental period (DIM) | P-value |  |  |  |
|--------------------------|-----------|---------------------------|---------|---|---|---|
|                          |           | P1 60                     | P1 120  | P2 190 | P3 230 | SEM | T  | DIM  | T × DIM |
| Milk protein             |           |                           |         |       |       |     |     |      |         |
| CSN1                     | CON       | 100                       | 423     | 356   | 397   | 40.5 | 0.77 | 0.62  | 0.75    |
|                          | POFA      | 100                       | 129     | 183   | 159   |       |      |       |         |
|                          | RSO       | 100                       | 338     | 428   | 261   |       |      |       |         |
|                          | SEM       | 39.4                      | 42.2    |       |       |       |      |       |         |
| CSN2                     | CON       | 100                       | 212     | 232   | 428   | 20.2 | 0.78 | <0.01 | 0.64    |
|                          | POFA      | 100                       | 166     | 241   | 342   |       |      |       |         |
|                          | RSO       | 100                       | 154     | 303   | 348   |       |      |       |         |
|                          | SEM       | 18.1                      |         |       |       |       |      |       |         |
| CSN3                     | CON       | 100                       | 467     | 482   | 567   | 21.1 | 0.95 | 0.04  | 0.31    |
|                          | POFA      | 100                       | 307     | 536   | 366   |       |      |       |         |
|                          | RSO       | 100                       | 442     | 801   | 441   |       |      |       |         |
|                          | SEM       | 30.8                      |         |       |       |       |      |       |         |
| LALBA                    | CON       | 100                       | 139     | 77    | 114   | 20.3 | 0.46 | 0.12  | 0.39    |
|                          | POFA      | 100                       | 130     | 96    | 101   |       |      |       |         |
|                          | RSO       | 100                       | 108     | 111   | 91    |       |      |       |         |
|                          | SEM       | 22.6                      |         |       |       |       |      |       |         |
| Apoptosis                | CASP8     | CON                       | 100     | 207   | 450   | 897   | 20.0 | 0.41 | <0.01 | 0.04    |
|                          | POFA      | 100                       | 165     | 457   | 354   |       |      |       |         |
|                          | RSO       | 100                       | 216     | 577   | 533   |       |      |       |         |
|                          | SEM       | 19.0                      |         |       |       |       |      |       |         |
| Lipid metabolism        | CD36      | CON                       | 100     | 111   | 296   | 582   | 22.1 | 0.68 | <0.01 | 0.56    |
|                          | POFA      | 100                       | 236     | 333   | 621   |       |      |       |         |
|                          | RSO       | 100                       | 181     | 511   | 598   |       |      |       |         |
|                          | SEM       | 21.9                      |         |       |       |       |      |       |         |
|                          | FABP3     | CON                       | 100     | 236   | 221   | 295   | 27.6 | 0.22 | 0.28  | 0.65    |
|                          | POFA      | 100                       | 316     | 563   | 472   |       |      |       |         |
|                          | RSO       | 100                       | 443     | 582   | 521   |       |      |       |         |
|                          | SEM       | 19.2                      |         |       |       |       |      |       |         |
|                          | FASN      | CON                       | 100     | 167   | 96    | 207   | 23.1 | 0.65 | 0.47  | 0.32    |
|                          | POFA      | 100                       | 168     | 151   | 178   |       |      |       |         |
|                          | RSO       | 100                       | 125     | 144   | 114   |       |      |       |         |
|                          | SEM       | 28.3                      |         |       |       |       |      |       |         |
|                          | G6PD      | CON                       | 100     | 135   | 306   | 38    | 53.5 | 0.78 | <0.01 | 0.77    |
|                          | POFA      | 100                       | 92      | 293   | 67    |       |      |       |         |
|                          | RSO       | 100                       | 129     | 183   | 65    |       |      |       |         |
|                          | SEM       | 12.9                      |         |       |       |       |      |       |         |
|                          | GPAM      | CON                       | 100     | 323   | 272   | 244   | 28.4 | 0.49 | 0.08  | 0.27    |
|                          | POFA      | 100                       | 252     | 546   | 362   |       |      |       |         |
|                          | RSO       | 100                       | 471     | 639   | 254   |       |      |       |         |
|                          | SEM       | 24.5                      |         |       |       |       |      |       |         |
|                          | LPL       | CON                       | 100     | 265   | 123   | 212   | 36.0 | 0.43 | 1.00  | 0.60    |
|                          | POFA      | 100                       | 390     | 441   | 422   |       |      |       |         |
|                          | RSO       | 100                       | 408     | 581   | 460   |       |      |       |         |
|                          | SEM       | 24.9                      |         |       |       |       |      |       |         |
|                          | SCD1      | CON                       | 100     | 244   | 369   | 896   | 24.9 | 0.60 | <0.01 | 0.62    |
|                          | POFA      | 100                       | 169     | 217   | 620   |       |      |       |         |
|                          | RSO       | 100                       | 101     | 263   | 476   |       |      |       |         |
|                          | SEM       | 22.4                      |         |       |       |       |      |       |         |
|                          | SCD5      | CON                       | 100     | 69    | 48    | 201   | 27.0 | 0.73 | <0.01 | 0.32    |
|                          | POFA      | 100                       | 90      | 85    | 170   |       |      |       |         |
|                          | RSO       | 100                       | 38      | 59    | 171   |       |      |       |         |
|                          | SEM       | 20.9                      |         |       |       |       |      |       |         |
| Transcription factor     | SREBF1    | CON                       | 100     | 305   | 538   | 129   | 42.9 | 0.56 | <0.01 | 0.78    |
|                          | POFA      | 100                       | 97      | 301   | 82    |       |      |       |         |
|                          | RSO       | 100                       | 338     | 377   | 88    |       |      |       |         |
|                          | SEM       | 23.6                      |         |       |       |       |      |       |         |
|                          | PPARG1    | CON                       | 100     | 412   | 517   | 198   | 27.6 | 0.43 | <0.01 | 0.55    |
|                          | POFA      | 100                       | 152     | 421   | 120   |       |      |       |         |

Continued
dance of 19 genes expressed over the lactation period, which is in agreement with their putative implications in milk component synthesis.

**Genes Involved in FA and Lipid Synthesis**

Over the experimental period, from 120 to 230 DIM, lactation stage induced no variation in mammary mRNA abundance of *LPL, FABP3, GPAM, or FASN* (Table 5). The result for *FASN* is not in accordance with the 3-fold decrease in FASN protein abundance reported in late versus early lactation in dairy cows (Mol et al., 2018). Similarly, a study in dairy sheep analyzing the expression of genes related to mammary gland fat metabolism suggested that genes involved in de novo fat synthesis in sheep milk show increased expression in late lactation (Suárez-Vega et al., 2016). In line with our data, Yadav et al. (2015), studying lipogenic gene expression in milk purified mammary epithelial cells (MEC) at different stages of lactation in buffalo, reported that *LPL* expression remained unchanged during lactation. The lack of changes over the experimental period (from 120 to 230 DIM) for *FASN* and *LPL*, despite observed variations in the milk fat yields of FA synthesized de novo or taken up by the mammary gland (Table 4), might be attributed to post-transcriptional or posttranslational regulation of these genes.

In accordance with metabolic changes that occur over a lactation period, mRNA abundances of *G6PD, SREBF1, and PPARG1* increased from 60 DIM and over the experimental period (from 120 to 200 DIM) before decreasing at 230 DIM (Table 5). These results are in line with previous data in dairy cows (Bionaz and Loor, 2008) and in milk purified MEC in buffalo (Yadav et al., 2015), reporting either increases throughout a lactation or increases and decreases at the end of lactation for mammary lipogenic genes. This is also in line with a recent functional analysis of the dairy cow mammary transcriptome between early lactation and the dry period, showing that lactation was supported by increased gene expression related to metabolic processes for milk component synthesis and nutrient transport (Lin et al., 2019).

The abundance of *CD36* mRNA (involved in long-chain FA transport) increased across the whole lactic
tation period, despite the decrease in milk fat yield observed at 230 DIM (Table 5). This result is in line with the observed variation in expression of 2 other genes involved in long-chain FA metabolism (transport and conversion to their active acyl-CoA forms, respectively), SLC27A and ACSL8, from −15 to 240 DIM in cows (Bionaz and Loor, 2008). When looking at gene expression variations from early lactation (90 DIM) to the dry period in dairy cows, upregulation of genes involved in FA transport process, including CD36 (Lin et al., 2019), has also been observed. Together, these data suggest that lactation is supported by increased gene expression related to nutrient transport, in particular for FA.

Similarly, mRNA abundance of SCD1 and SCD5, which are involved in FA Δ-9 desaturation, increased at the end of the experimental period (230 DIM), after previously showing stable expression throughout lactation (Table 5). This result is consistent with the stable expression of these genes over a lactation observed in milk purified MEC in buffalo (from 15 to 240 DIM; Yadav et al., 2015) and with their ranking in the top 50 upregulated transcripts in the mammary glands of dairy cows during early lactation (90 DIM) versus the dry period (Lin et al., 2019). It is also consistent with the role of the corresponding proteins in the endogenous synthesis of UFA that contributes to the fluidity of the milk fat and cell membranes, regardless of the tissue. Indeed, these genes are ubiquitously highly expressed in the major animal body tissues, particularly in the lactating mammary gland (Lengi and Corl, 2007; Toral et al., 2013). In the present study and the larger work presented by our group (Inglingstad et al., 2017), mRNA abundance of SCD1 was positively correlated with the milk desaturation ratio 14:1 cis-9/14:0 (r = 0.46; P < 0.001, n = 108), which is in line with the reported close relationship between this milk FA desaturation ratio and stearoyl-CoA desaturase (SCD) activity in goats (Bernard et al., 2008).

Finally, mRNA abundance of MFGE8 and XDH, which encode proteins associated with milk fat, remained stably expressed over the lactation period (Table 5), in line with their implied role in the formation of lipid droplets. This result is in accordance with expression data for XDH across 3 lactation stages in MEC in buffalo (Arora et al., 2019) and for glyceral-1, another protein of the milk fat globule membrane, across 4 lactation stages in dairy ewes (Suárez-Vega et al., 2016). In the present study and the larger work presented by our group (Inglingstad et al., 2017), XDH mRNA abundance was negatively correlated with milk LPL activity (r = −0.51; P < 0.001, n = 108), which suggests a relationship between XDH in the fat globule membrane and the effectiveness of the membrane barrier with respect to LPL, in addition to the role of XDH in mammary cell lipid secretion (Mather et al., 2019).

**Groups of Genes Involved in Milk Protein Synthesis**

Protein metabolism is a major biological process in all lactation stages. Accordingly, mRNA abundance of CSN1S1, CSN2, CSN1S1, CSN3, and LALBA was stable over the lactation period (Table 5) in the present study, which is relevant for the synthesis of milk proteins (Table 3). Indeed, it was previously established that the genes encoding the main milk proteins, casein and whey, were expressed abundantly throughout lactation in goats (Crisà et al., 2016), in 2 breeds of ewes (Assaf and Churra; Suárez-Vega et al., 2016), in 2 breeds of cows (Jersey and Kashmiri; Bhat et al., 2019), and in buffaloes (Sharma et al., 2019). High expression of the MTOR gene, which activates protein synthesis, and of genes that encode AA transporters in yak mammary tissues throughout lactation was reported previously (Xia et al., 2018). Although most of the milk protein and milk protein-related genes were highly expressed across all 3 stages, the early stage is mainly defined by high protein metabolism and the corresponding gene expression, which then decline in mid and late stages in caprine and bovine species (Crisà et al., 2016; Arora et al., 2019; Lin et al., 2019).

Despite the decrease in lactose and fat yields over lactation, the expression pattern of the glucose transporter GLUT4 (SLC2A4, solute carrier family 2 member 4) increased from 120 to 230 DIM, as did GLUT1 (SLC2A1, solute carrier family 2 member 1) during the same lactation period for in milk enriched MEC of water buffalo (Yadav et al., 2014). A similar pattern was observed for 3 glucose transporters (SLC2A1, SLC2A3, and SLC2A8) in yak mammary tissue during a lactation cycle (Xia et al., 2018). These discrepancies between production and gene expression data have not yet been investigated and could be explained by post-transcriptional regulation of these genes.

**Genes Involved in Cell Differentiation**

Over the lactation period, rates of milk synthesis and yields of milk constituents by the mammary gland are mainly determined by the balance between proliferation and apoptosis of MEC during lactation (Boutinaud et al., 2004). Indeed, from the beginning of lactation to the peak, the number and activity of MEC increase to support milk production and then decrease at the end of lactation when the mammary tissue undergoes involution (Watson, 2006). In the present study, we investigated the expression of the genes CASP8 and ELF3, which are involved in cell apoptosis and mammary
growth and involution; mRNA abundance of these 2 genes increased over the experimental period to reach a maximum at 230 DIM. The observed variations in expression of ELF3 are in accordance with a mammary transcriptome analysis between early lactation and dry period in dairy cows, showing upregulation of genes involved in mammary tissue remodeling pathways (Lin et al., 2019). Similarly, characterization of the sheep milk transcriptome over a lactation period (on d 10, 50, 120, and 150) demonstrated an increase in the expression of a few genes involved in mammary involution pathways at 150 DIM compared with 10 DIM (Suárez-Vega et al., 2016).

A decline in milk yield during late lactation is generally attributed to the loss of secretory MEC due to apoptosis (Boutinaud et al., 2004). Consistent with this, CASP8 mRNA abundance at 230 DIM increased in goat mammary tissue, as was also found for BAX and BCL2 expression in milk MEC from buffalo at 240 DIM (Yadav et al., 2014). Similarly, studies in dairy cows reported both high cell apoptosis and proliferation in mammary gland due to high remodeling of this tissue during early lactation (Capuco et al., 2001; Nørgaard et al., 2008).

CONCLUSIONS

This is the first study to describe the expression of 19 mammary genes over a complete lactation in goats; our results provide information of candidate genes and pathways involved in different stages of lactation in goats fed lipid-supplemented diets. We observed no variation in mammary mRNA of lipogenic genes caused by the diets, although POFA increased milk fat content and yield, and POFA and RSO affected milk FA composition. Our data suggest that other factors (e.g., substrate availability for mammary metabolism or posttranscriptional or posttranslational regulation of these genes) could play important roles in responses of milk fat and FA to changes in diet composition in the goat. We found a significant effect of lactation stage on milk composition and mRNA abundance of genes involved in milk protein and fat synthesis. According to previous data in dairy cows, yak, and buffaloes, over the course of lactation, synthesis of milk components is supported by increased expression of genes related to nutrient transport, desaturation (SCD genes), and casein and whey proteins. The late-lactation period was characterized by increased expression of genes involved in apoptosis and tissue remodeling. We conclude that in goats, mammary gene expression is more regulated by stage of lactation than by lipid supplementation.

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