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Comparison of the nutritional regulation of milk fat secretion and composition in cows and goats

P. G. Toral,*† Y. Chilliard,*† J. Rouel,*† H. Leskinen,‡ K. J. Shingfield,‡§ and L. Bernard*†[1]
*INRA, UMR1213 Herbivores, F-63122 Saint-Genès-Champanelle, France
†Clermont Université, VetAgro Sup, UMR Herbivores, BP 10448, F-63000, Clermont-Ferrand, France
‡Natural Resources Institute Finland (Luke), Green Technology, Nutritional Physiology FI-31600, Jokioinen, Finland
§Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, SY23 3FL, United Kingdom

ABSTRACT

A study with 2 ruminant species (goats and cows) with inherent differences in lipid metabolism was performed to test the hypothesis that milk fat depression (MFD) due to marine lipid supplements or diets containing high amounts of starch and plant oil is caused by different mechanisms and that each ruminant species responds differently. Cows and goats were allocated to 1 of 3 groups (4 cows and 5 goats per group) and fed diets containing no additional oil (control) or supplemented with fish oil (FO) or sunflower oil and wheat starch (SOS) according to a 3 × 3 Latin square design with 26-d experimental periods. In cows, milk fat content was lowered by FO and SOS (−31%), whereas only FO decreased milk fat content in goats (−21%) compared with the control. Furthermore, FO and SOS decreased milk fat yield in cows, but not in goats. In both species, FO and SOS decreased the secretion of <C16 and C16 fatty acids (FA), and FO lowered >C16 FA output. However, SOS increased milk secretion of >C16 FA in goats. Compared with the control, SOS resulted in similar increases in milk *trans*-10,*cis*-12 conjugated linoleic acid (CLA) in both species, but caused a 2-fold larger increase in *trans*-10 18:1 concentration in cows than for goats. Relative to the control, responses to FO in both species were characterized by a marked decrease in milk concentration of 18:0 (−74%) and *cis*-9 18:1 (−62%), together with a ~5-fold increase in total *trans* 18:1, but the proportionate changes in *trans*-10 18:1 were lower for goats. Direct comparison of animal performance and milk FA responses to FO and SOS treatments demonstrated interspecies differences in mammary lipogenesis, suggesting a lower sensitivity to the inhibitory effects of *trans*-10,*cis*-12 CLA in goats and that ruminal biohydrogenation pathways are more stable and less prone to diet-induced shifts toward the formation of *trans*-10-containing intermediates in goats compared with cows. Even though a direct cause and effect could not be established, results suggest that regulation of milk fat synthesis during FO-induced MFD may be related to a shortage of 18:0 for endogenous mammary *cis*-9 18:1 synthesis, increase in the incorporation of *trans* FA in milk triacylglycerols, and limitations in the synthesis of FA de novo to maintain milk fat melting point. However, the possible contribution of biohydrogenation intermediates with putative antilipogenic effects in the mammary gland, including *trans*-9,*cis*-11 CLA, *trans*-10 18:1, or *cis*-11 18:1 to FO-induced MFD cannot be excluded.
Key words: cow, goat, milk fatty acid, milk fat depression

INTRODUCTION

Milk fat synthesis represents a major energy cost for milk production and plays a central role in determining dairy product quality and the partitioning of energy into milk. Transfer of dietary FA into milk fat is energetically favorable compared with FA synthesis de novo (Moe, 1981). Depending on payment scheme, economic advantages may exist for producing milk with a specific fat content, whereas legal limits on the minimum amount of fat in whole milk are imposed in numerous countries. For these reasons, there has been considerable interest for more than 3 decades in understanding the influence of diet on the regulation of milk fat secretion and FA composition and identifying the causes of diet-induced milk fat depression (**MFD**; Palmquist and Jenkins, 1980; Bauman and Griinari, 2003; Shingfield et al., 2010).

Several theories have been proposed to explain the causes of MFD, with most found to be inadequate or

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[1]Corresponding author: laurence.bernard@clermont.inra.fr

incomplete (Bauman and Griinari, 2003; Shingfield and Griinari, 2007). The biohydrogenation (**BH**) theory appears to be the most universal, and it attributes diet-induced MFD to an inhibition of mammary lipogenesis by specific FA intermediates formed in the rumen on certain diets as a consequence of alterations in ruminal BH pathways (Bauman and Griinari, 2003). *Trans*-10,*cis*-12 CLA is the only BH intermediate shown unequivocally to inhibit milk fat synthesis, but additional BH intermediates including *cis*-10,*trans*-12 CLA, *trans*-9,*cis*-11 CLA, and possibly *trans*-10 18:1, as well as other mechanisms, may also be involved (Harvatine et al., 2009; Shingfield et al., 2010). Although the BH theory provides a basis for explaining most cases of MFD on starch-rich diets or plant oil in cows, direct inhibition by the BH intermediates with confirmed or putative antilipogenic effects (*trans*-10,*cis*-12 CLA, *cis*-10,*trans*-12 CLA, and *trans*-9,*cis*-11 CLA) does not, in isolation, explain MFD in cows or sheep fed diets containing marine oils (Loor et al., 2005a; Gama et al., 2008; Toral et al., 2010). To accommodate these findings, Shingfield and Griinari (2007) proposed an extension of the BH theory to include the role of changes in the availability of preformed long-chain FA to the mammary gland. Several reports have suggested that a shortage of 18:0 for endogenous *cis*-9 18:1 synthesis in the mammary gland, together with an increase in the supply of *trans* FA formed in the rumen, would increase milk fat melting point, exceeding the capacity to maintain milk fat fluidity and thereby lower the rate of fat removal in mammary epithelial cells (Loor et al., 2005a; Gama et al., 2008). This phenomenon may offer an explanation for MFD in ewes offered supplements of fish oil or marine algae, whereas high-starch diets, plant oil, and oilseed supplements do not alter milk fat content in this species (Shingfield et al., 2010; Toral et al., 2010). However, milk production in goats is characterized by an absence of diet-induced MFD, even on diets containing high amounts of starch and plant oil (Chilliard et al., 2003; Martínez Marín et al., 2012; Bernard et al., 2009) or in response to dietary fish oil supplements (Toral et al., 2014).

The reasons for the differential lipogenic responses between ruminant species are not well understood, but based on indirect comparisons of milk FA composition, have been suggested to reflect differences in ruminal BH and mammary lipid metabolism (Chilliard et al., 2007, 2014; Shingfield et al., 2010). However, no direct interspecies comparisons of diet-induced MFD have been reported in the literature.

A comparative study with lactating cows and goats presenting differences in their susceptibility to diet-induced MFD was undertaken to test the hypotheses that MFD due to marine lipid supplements or a diet containing high amounts of starch and plant oils is caused by distinct mechanisms and that mammary lipogenic responses differ between ruminant species. To meet these objectives, cows and goats were fed a basal diet containing no additional lipid (control), a similar diet supplemented with fish oil (**FO**), or a diet containing additional starch and sunflower oil (**SOS**). Changes in milk production, fat yield, and milk FA were measured and used to infer possible mechanisms responsible for differences in the regulation of mammary lipogenesis due to diet and ruminant species.

MATERIALS AND METHODS

Animals, Experimental Design, Diets, and Management

All experimental procedures were approved by the Animal Care Committee of INRA in accordance with the guidelines established by the European Union Directive 2010/63/EU. Twelve Holstein cows and 15 Alpine goats, all multiparous, nonpregnant, and at similar lactation stage (67 ± 6.5 and 73 ± 1.4 DIM for cows and goats, respectively) were used. Cows and goats were housed in individual stalls in separate dedicated facilities at the same research site. Animals were then allocated to 1 of 3 groups (4 cows and 5 goats per group) that were balanced according to DIM, milk production, milk fat content, parity, and the genotype score at the αS1-CN locus for goats, and used in a replicated 3 × 3 Latin square to test the effects of 3 treatments during three 26-d experimental periods (Kaps and Lamberson, 2009) from April to June 2012. Unfortunately, 1 goat had to be withdrawn from the experiment due to diarrhea.

All animals were offered grass hay ad libitum supplemented with concentrates containing no additional lipid (control), fish oil (**FO**; anchovy oil, SA Daudruy Van Cauwenberghe et Fils, Dunkerque, France) or sunflower oil (Auvergne Trituration, Lezoux, France) and wheat starch (**SOS**; Table 1). Both fish oil and sunflower oil (stored in the dark at room temperature) were mixed manually with other ingredients immediately before feeding out and fed in amounts to supply 420 and 1,000 g of oil/d in cows and 50 and 120 g of oil/d for goats, respectively. Diets were offered as 2 equal meals at 0830 and 1600 h. Hay and concentrate 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 (40:60 on a DM basis). Before starting the experiment, all animals received the control diet during a 28-d adaptation period. Animals had access to a constant supply of fresh water and were milked at 0800 and 1530 h.

Table 1. Formulation of experimental concentrates and chemical composition of concentrates and grassland hay[1]

Item	Concentrate[1]			Grassland hay
	Control	FO	SOS	
Ingredients, g/kg of DM				
Pelleted dehydrated alfalfa	294	257	—	—
Cracked corn grain	549	547	377	—
Flattened wheat grain	—	—	374	—
Soybean meal	143	144	144	—
Fish oil[2]	—	36.4	—	—
Sunflower oil[3]	—	—	89.7	—
Mineral and vitamin premix[4]	14.0	15.6	15.3	—
Chemical composition, g/kg of DM				
OM	933	936	963	920
CP	181	174	159	90
NDF	214	198	112	569
ADF	119	108	35	314
Starch	401	399	541	—
Ether extract	30	66	113	15
14:0	0.05	3.45	0.09	0.10
16:0	4.62	11.72	9.58	2.12
cis-9 16:1	0.04	3.22	0.12	0.04
18:0	0.66	1.99	3.29	0.21
cis-9 18:1	7.06	10.36	28.48	0.52
cis-11 18:1	0.23	1.44	0.22	0.07
18:2n-6	14.6	15.0	69.9	1.38
18:3n-3	2.22	2.51	0.86	2.36
20:5n-3	ND[5]	6.68	ND	ND
22:5n-3	ND	0.69	ND	ND
22:6n-3	ND	2.79	ND	ND
Total FA	30.1	65.9	114.3	7.6
Energy,[6] MJ/kg of DM	7.61	8.11	9.53	4.84
Protein, g of PDI[7]/kg of DM	123	119	114	56

[1]Control = basal concentrate containing no additional oil; FO = basal concentrate supplemented with fish oil; SOS = basal concentrate containing sunflower oil and wheat starch.

[2]Anchovy oil (SA Daudruy Van Cauwenbergheet Fils, Dunkerque, France) contained (g/kg of total FA): 12:0 (2.21), 14:0 (93.5), 15:0 (5.70), 16:0 (199), *cis*-9 16:1 (87.3), 17:0 (5.04), 18:0 (37.0), *cis*-9 18:1 (91.8), *cis*-11 18:1 (33.4), 18:2n-6 (16.2), 18:3n-6 (2.48), 18:3n-3 (13.6), 20:0 (4.37), *cis*-11 20:1 (1.60), 20:2n-6 (1.31), 20:3n-6 (1.62), 20:4n-6 (12.4), 20:5n-3 (183), 22:0 (1.25), 22:5n-3 (19.0), 22:6n-3 (76.7), 24:0 (2.04), *cis*-15 24:1 (2.72), and total FA (952 g/kg).

[3]Sunflower oil (Auvergne Trituration, Lezoux, France) contained (g/kg of FA): 16:0 (63.3), 18:0 (31.7), *cis*-9 18:1 (255), 18:2n-6 (630), 18:3n-3 (1.48), 20:0 (2.17), 22:0 (6.52), 24:0 (2.59), and total FA (953 g/kg).

[4]Mineral and vitamin premix (Groupe Centre-Lait, Aurillac, France) declared as containing: Ca (200 g/kg), P (25 g/kg), Mg (45 g/kg), Na (35 g/kg), Zn (6 g/kg), Mn (3.5 g/kg), Cu (1.3 g/kg), vitamin A (400,000 IU/kg), vitamin D_3 (130,000 IU/kg), and vitamin E (1,600 mg/kg).

[5]nd = not detected.

[6]Net energy for lactation calculated according to INRA (1989).

[7]PDI (protein digestible in the intestine) calculated according to INRA (1989).

Measurements and Sampling Procedures

Individual feed intake was recorded daily, but only measurements collected during the last 3 d of each experimental period were used for statistical analysis. Representative samples of hay and concentrates were collected weekly. A subsample was used to determine DM content by drying at 103°C for 48 h. Weekly subsamples were composited by period and species and submitted for the determination of chemical composition.

Milk yields of individual animals were recorded over 4 consecutive milkings starting at 0800 h on d 23 of each experimental period. With the same frequency, samples of milk for the measurement of fat, protein, and lactose were collected individually and treated with preservative (bronopol-B2; Trillaud, Surgères, France). Samples of unpreserved milk were also collected over 2 consecutive milkings from 0800 h on d 24 of each experimental period, stored at −20°C, composited according to milk yield, and submitted for FA analysis. Additional samples of unpreserved milk were collected and stored at −20°C until analyzed for lipoprotein lipase (**LPL**) activity (Bernard et al., 2005). Blood samples (10 mL) from the jugular vein were collected in evacuated collection tubes containing EDTA (10 mL;

BD Vacutainer, Plymouth, UK) from all experimental animals immediately before morning feeding on d 26 of each experimental period. Plasma recovered after centrifugation (1,500 × *g* for 15 min at 4°C) was stored at −20°C until submitted for the determination of metabolite and hormone concentrations. For all animals, BW was measured on d 24 of each experimental period.

Chemical Analysis

Chemical composition of feed ingredients was determined using standard procedures (AOAC International, 1997). Fatty acid methyl esters of lipid in feed samples were prepared using a one-step extraction-transesterification procedure (Sukhija and Palmquist, 1988) using 23:0 (Sigma, Saint-Quentin Fallavier, France) as an internal standard. The FAME recovered were quantified using a GC (Trace-GC 2000 Series, Thermo Finnigan, Les Ulis, France) equipped with a flame ionization detector and a 100-m fused silica capillary column (0.25 mm i.d.) coated with a 0.2-μm film of cyanopropylpolysiloxane (CP-Sil 88; Chrompack Nederland BV, Middelburg, the Netherlands) and a temperature gradient program (Loor et al., 2005b), using hydrogen as the carrier and fuel gas, operated at constant pressure (147 kPa).

Milk fat, true protein, and lactose contents were determined by mid-infrared spectrophotometry using a Milkoscan 4000 (Foss Electric, Hillerød, Denmark) calibrated using historical samples of bovine and caprine milk for which reference measurements had been made (AOAC International, 1997). Total lipid in 100 mg of freeze-dried milk was converted to FAME by incubation with 2 mL of 0.5 *M* sodium methoxide in anhydrous methanol and 1 mL of hexane at 50°C for 15 min. After cooling, 1 mL of methanol and hydrochloric acid (95:5 vol/vol) was added to the reaction mixture and incubated at 50°C for 15 min. Methyl esters were recovered in 1.5 mL of hexane, washed with 3 mL of aqueous (6% wt/wt) potassium carbonate, and analyzed using a GC (Agilent 7890A GC System, Santa Clara, CA) equipped with a CP-Sil 88 capillary column. The profile of FAME in a 2-μL sample at a split ratio of 1:50 was determined using a temperature gradient program (Loor et al., 2005b), with hydrogen as the carrier and fuel gas, operated at constant pressure (147 kPa). Isomers of 18:1 were further resolved in an additional analysis under the same conditions, with the exception that a smaller sample volume (0.6-μL) was injected onto the column. Peaks were routinely identified based on retention time comparisons with commercial authentic standards (NCP #463, Nu-Chek Prep Inc., Elysian, MN; Supelco #37, Supelco Inc., Bellefonte, PA; L8404 and O5632; Sigma). Methyl esters not available as commercial standards were identified based on GC-MS analysis of 4,4-dimethyloxazoline (**DMOX**) derivatives in positive electron ionization mode, prepared from selected samples of milk FAME, using a GC (model 6890, Hewlett-Packard, Wilmington, DE) equipped with a quadrupole mass spectrometer (model 5973N, Agilent Technologies Inc., Wilmington, DE). Preparation of DMOX derivatives, parameters and conditions used for GC-MS analysis, and interpretation of mass spectra were in accordance with earlier reports (Halmemies-Beauchet-Filleau et al., 2011). Identification was further verified based on retention time and elution order comparisons with samples of milk fat from cows fed fish oil analyzed by complementary silver-ion thin-layer chromatography and GC-MS analysis of fractionated FAME and corresponding DMOX derivatives (Kairenius et al., 2015). The distribution of CLA isomers in milk samples was determined using a HPLC system (model 1090; Hewlett-Packard) equipped with 4 silver-impregnated silica columns (ChromSpher 5 lipids, 250 × 4.6 mm, 5 μm particle size; Agilent Technologies Inc.) coupled in series. Methyl esters of CLA were separated under isothermal conditions at 22°C using 0.1% (vol/vol) acetonitrile in heptane at a flow rate of 1 mL/min and monitoring column effluent at 233 and 210 nm (Halmemies-Beauchet-Filleau et al., 2011).

Milk FA composition was expressed as a weight percentage of total FA using theoretical relative response factors (Wolff et al., 1995). Concentrations of CLA isomers were calculated based on proportionate peak area responses determined by HPLC and the sum of *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA, and *cis*-9,*trans*-11 CLA weight percentage determined by GC analysis.

Plasma glucose and NEFA concentrations were determined by spectrophotometry using methods based on glucose dehydrogenase (Glucose RTU kit; BioMérieux, Lyon, France) and acyl-CoA synthetase (Wako NEFA HR2 kit; Oxoid, Dardilly, France), respectively. Concentrations of BHBA were measured according to Brashear and Cook (1983), and insulin, IGF-I, and leptin determined by RIA using commercial kits (Insulin CT kitCIS, Bio International, Gif-sur-Yvette, France; IGF-I RIA-CT, Medianost GmbH, Reutlingen, Germany) or a specific assay (Delavaud et al., 2000).

Calculations and Statistical Analysis

Calculations and statistical analysis were performed on measurements made on 12 cows and 14 goats due to the removal of 1 goat early in the experiment. Milk FA melting point was calculated as the sum of melting points for constituent FA weighted by their respective

molar percentages (Jensen and Patton, 2000). For each individual FA, the melting point was obtained from Gunstone et al. (1994) and, failing that, from the Lipid Bank database (http://www.lipidbank.jp). For FA not reported in the literature, melting point was estimated on the basis of structural similarities with isomers of known melting point. Apparent transfer of 20:5n-3, 22:5n-3, and 22:6n-3 from FO into milk were calculated as [grams of milk FA yield × (% FA in milk fat − % in control milk fat)/(DMI × % FA in the diet)] × 100.

All data were subjected to ANOVA for a 3 × 3 Latin square design (Kaps and Lamberson, 2009) using the MIXED procedure of the SAS (version 9.2, SAS Institute Inc., Cary, NC). The statistical model included the fixed effects of period, species, experimental diet, and their interaction, as well as the order in which treatments were allocated to each animal and the random effect of animal nested within treatment order. For FA found exclusively in milk of cows and goats fed the FO treatment, data were analyzed by the same model, with the exception that fixed effects due to diet and diet by species interaction were removed. Differences between means were tested based on least square differences using the default pairwise *t*-test in the pdiff option in the lsmeans statement, and declared significant at $P < 0.05$. *P*-values >0.05 and ≤0.10 were interpreted as a trend toward significance.

RESULTS

Diet Composition

Formulation of experimental concentrates and the chemical composition and FA profile of concentrate supplements and grassland hay are reported in Table 1. By design, dehydrated alfalfa pellets and a proportion of cracked corn in the control concentrate supplement were replaced by flattened wheat to increase the starch content of the SOS treatment. Furthermore, the inclusion of oil resulted in a higher ether extract, total FA, and energy content of the FO and SOS concentrates relative to the control. The energy content of the control concentrate was increased by approximately 7 and 25% on the FO and SOS treatments, respectively, whereas digestible protein in the intestine was marginally decreased (−7%) for SOS compared with the control concentrate (Table 1). Relative to the control, the addition of fish oil increased concentrations of 14:0, 16:0, *cis*-9 16:1, and *cis*-11 18:1 and the appearance of 20:5n-3, 22:5n-3, and 22:6n-3 in the FO concentrate, whereas the inclusion of sunflower oil resulted in the SOS diet containing greater amounts of 18:0, *cis*-9 18:1, and 18:2n-6 (Table 1).

Animal Performance

Effect of treatments on animal performance and milk composition are reported in Table 2. Daily DMI, milk yield, and milk fat outputs expressed per unit of BW for both species are shown in Figure 1. On the control treatment, DMI per kilogram of BW was 10.7% higher ($P = 0.028$) in goats compared with cows (Figure 1). However, milk yield per kilogram of BW tended ($P = 0.096$) to be lower (−13%) for goats than cows fed the control (Figure 1). Milk fat and lactose contents were similar between species on the control, whereas milk protein content was higher ($P < 0.001$) for goats than cows for all diets (on average, 19.8%; Table 2). Daily milk yields of C16 and >C16 FA, expressed as millimole per kilogram of BW, were lower in goats than cows fed the control treatment ($P < 0.05$), but no differences ($P = 0.39$) were observed for the secretion of <C16 FA (Figure 1).

By design, grass hay was fed ad libitum and the amount of concentrate offered was adjusted daily to maintain the target dietary forage to concentrate ratio (40:60; on a DM basis); therefore, only small variations in this ratio based on the actual amounts of feed ingredients consumed were observed for the control, FO, and SOS treatments (42:58, 40:60, and 40:60, respectively). Throughout the trial, fish oil and sunflower oil supplements were fed at a fixed rate of 420 and 1,000 g oil/d in cows and 50 and 120 g oil/d for goats, respectively. However, despite controlling the amounts of feeds offered, the amount of sunflower oil as a function of total DMI differed between cows and goats (50 and 57 g/kg of DM, respectively). Fish oil represented, on average, 22 g/kg of total DMI for both species.

Inclusion of oil supplements decreased DMI ($P < 0.001$) compared with the control, the extent of which was similar in both species (mean response, −13%), whereas diet had no effect ($P = 0.81$) on daily milk yield expressed per kilogram of BW (Figure 1). Compared with the control, both FO and SOS increased ($P = 0.003$) milk lactose content in goats (Table 2). However, FO and SOS lowered ($P < 0.001$) milk fat content in cows (mean response, −31% compared with the control), whereas FO decreased ($P < 0.001$) milk fat content in goats (mean response, −21% relative to the control). Percentage decreases in milk fat yield were of a similar magnitude to that for milk fat content but the response to FO in goats was only significant when expressed on a kilogram of BW basis (Figure 1). Changes in the daily secretion of short- and medium-chain FA (4- to 15-carbon; <C16), C16, and long-chain FA (>C16) expressed as millimole per kilogram of BW are shown in Figure 1. In goats, FO and SOS lowered ($P < 0.001$) the yields of <C16 and 16-carbon FA (on

Table 2. Effect of dietary supplements of fish oil or sunflower oil and starch on intake, milk production, and milk lipoprotein lipase activity in cows and goats[1]

	Cows			Goats				*P*-value[3]		
Item	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
Intake, kg/d										
Grassland hay	10.19[a]	8.52[b]	8.08[b]	1.05[c]	0.90[c]	0.85[c]	0.32	<0.001	<0.001	<0.001
Concentrate	13.81[a]	12.21[b]	11.89[b]	1.46[c]	1.36[c]	1.29[c]	0.30	<0.001	<0.001	<0.001
Total DM	24.00[a]	20.73[b]	19.97[b]	2.49[c]	2.24[c]	2.17[c]	0.57	<0.001	<0.001	<0.001
OM	22.25[a]	19.26[b]	18.88[b]	2.34[c]	2.10[c]	2.03[c]	0.54	<0.001	<0.001	<0.001
CP	3.41[a]	2.91[b]	2.62[c]	0.36[d]	0.32[d]	0.28[d]	0.08	<0.001	<0.001	<0.001
NDF	8.75[a]	7.27[b]	5.94[c]	0.92[d]	0.79[d]	0.63[d]	0.23	<0.001	<0.001	<0.001
Starch	5.52[b]	4.88[c]	6.47[a]	0.59[d]	0.54[d]	0.70[d]	0.16	<0.001	<0.001	<0.001
FA intake, g/d										
14:0	1.7[c]	40.7[a]	1.8[c]	0.2[d]	4.8[b]	0.2[d]	0.05	<0.001	<0.001	<0.001
16:0	85.4[c]	156.4[a]	126.9[b]	9.2[f]	18.0[d]	14.5[e]	1.92	<0.001	<0.001	<0.001
cis-9 16:1	1.0[d]	37.5[a]	1.7[c]	0.1[f]	4.4[b]	0.2[e]	0.03	<0.001	<0.001	<0.001
18:0	11.2[c]	25.2[b]	38.6[a]	1.2[f]	2.9[e]	4.5[d]	0.25	<0.001	<0.001	<0.001
cis-9 18:1	102.7[c]	128.9[b]	325.5[a]	11.4[f]	15.0[e]	38.0[d]	2.32	<0.001	<0.001	<0.001
cis-11 18:1	3.9[b]	17.5[a]	3.3[c]	0.4[e]	2.1[d]	0.4[e]	0.09	<0.001	<0.001	<0.001
18:2n-6	215.1[b]	195.1[c]	799.5[a]	23.9[e]	22.7[e]	93.7[d]	5.13	<0.001	<0.001	<0.001
18:3n-3	54.6[a]	50.2[b]	29.2[c]	5.5[d]	5.4[d]	3.1[e]	1.10	<0.001	<0.001	<0.001
20:5n-3	ND[4]	77.1	ND	ND	9.1	ND	0.02	<0.001	—	—
22:5n-3	ND	8.0	ND	ND	0.9	ND	<0.01	<0.001	—	—
22:6n-3	ND	32.2	ND	ND	3.8	ND	0.01	<0.001	—	—
Total FA	491.9[c]	845.0[b]	1352.4[a]	53.5[f]	98.1[e]	157.6[d]	11.05	<0.001	<0.001	<0.001
Yield										
Milk, kg/d	29.8	30.3	29.6	2.46	2.45	2.46	1.015	<0.001	0.801	0.789
Fat, g/d	992[a]	706[b]	673[b]	77[c]	62[c]	74[c]	43.0	<0.001	<0.001	<0.001
True protein, g/d	833	839	875	81	82	86	32.0	<0.001	0.242	0.393
Lactose, g/d	1,513	1,525	1,491	120	126	129	53.9	<0.001	0.878	0.809
Concentration, g/100 g										
Fat	3.34[a]	2.34[c]	2.29[c]	3.11[ab]	2.47[c]	2.90[b]	0.188	0.294	<0.001	<0.001
True protein	2.80	2.77	2.96	3.37	3.39	3.46	0.101	<0.001	0.004	0.359
Lactose	5.08[ab]	5.04[ab]	5.04[ab]	4.93[b]	5.07[a]	5.16[a]	0.081	0.969	0.062	0.003
Milk LPL[5] activity, nmol/min per mL	531[a]	475[b]	510[a]	430[c]	273[e]	349[d]	12.6	<0.001	<0.001	<0.001
BW, kg	617[a]	596[b]	610[a]	59[c]	58[c]	58[c]	11.7	<0.001	0.002	0.005
Energy balance,[6] %	115	114	127	130	133	133	6.3	0.008	0.080	0.212
Protein balance,[7] %	155	148	130	167	162	132	8.4	0.177	<0.001	0.379

[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; FO = basal diet supplemented with fish oil; SOS = basal diet containing sunflower oil and wheat starch.

[2]SED = standard error of the difference.

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

[4]nd = not detected.

[5]LPL = lipoprotein lipase.

[6]Net energy for lactation balance (MJ/d) calculated according to INRA (1989) and expressed as a percent of estimated requirements.

[7]Protein balance (g PDI/d) calculated according to INRA (1989) and expressed as a percent of estimated requirements.

average −27%), but the decrease on the SOS treatment was partially alleviated by an increase (56%; $P < 0.001$) in the secretion of >C16 FA. Similarly, both FO and SOS lowered the yields of <C16 and C16 FA in cows, but the greater decreases in these FA on the SOS treatment (on average, −55%; $P < 0.001$) were not compensated for by an increase in the output of >C16 FA. Secretion of >C16 FA in milk was decreased by FO in both species (Figure 1).

Irrespective of diet, milk LPL activity was higher ($P < 0.001$) in cows than in goats, but the change in response to diet was substantially higher in goats (Table 2). For goats, FO and SOS decreased ($P < 0.001$) milk LPL activity by −37 and −19%, respectively, whereas FO only lowered (−11%) activity in bovine milk.

Energy and protein balances (INRA, 1989) were positive on all treatments for both species. Relative to the control, SOS tended ($P = 0.08$) to increase energy balance (+10%) in cows, but decreased ($P < 0.001$) protein balance in cows and goats (mean −19%). In cows, FO resulted in a marginal decrease ($P < 0.01$) in BW (−3%) compared with the control.

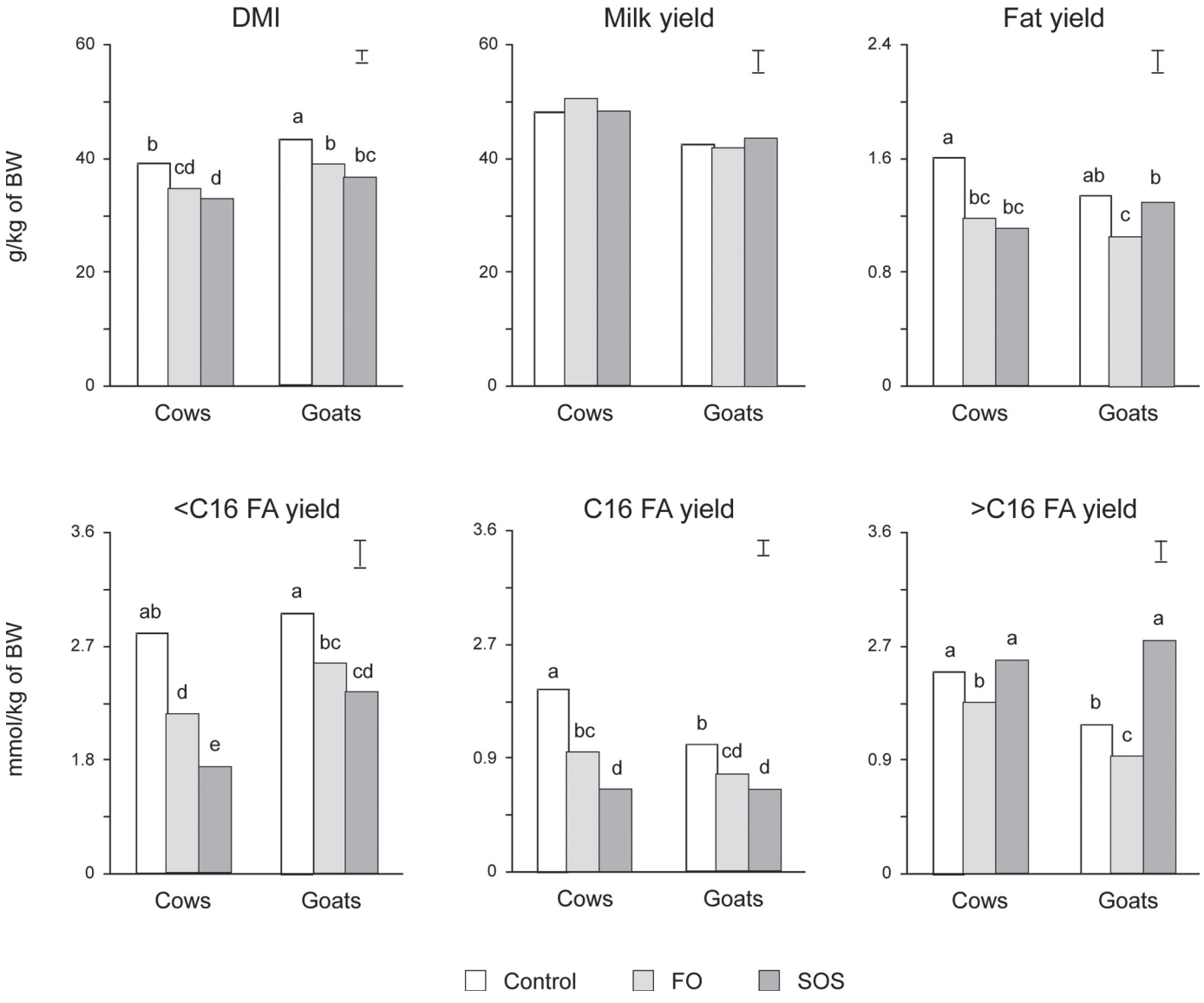

Figure 1. Daily DMI and the yields of milk, milk fat, and major groups of FA expressed on a kilogram of BW basis in cows and goats fed a basal diet containing no additional lipid (control), or supplemented with fish oil (FO) or sunflower oil and wheat starch (SOS). Within each parameter, values not sharing a common letter (a–e) differ at $P < 0.05$. Vertical bars indicate standard error of the difference. The group of FA <C16 represents FA synthesized de novo, those of >C16 represent preformed FA taken up from circulation, and C16 FA are derived from both sources.

Table 3. Effect of dietary supplements of fish oil or sunflower oil and starch on milk FA composition in cows and goats[1]

	Cows			Goats				*P*-value[3]		
FA, g/100 g of FA	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
4:0	3.39[a]	3.86[a]	2.57[b]	2.43[b]	2.27[b]	2.37[b]	0.262	<0.001	0.004	0.002
6:0	2.38[a]	1.95[b]	1.24[c]	2.50[a]	2.49[a]	2.06[b]	0.114	<0.001	<0.001	<0.001
8:0	1.30[c]	1.00[d]	0.61[e]	2.87[a]	3.00[a]	2.09[b]	0.120	<0.001	<0.001	<0.001
10:0	2.80[c]	2.15[d]	1.37[e]	10.12[a]	10.40[a]	6.32[b]	0.357	<0.001	<0.001	<0.001
cis-9 10:1	0.27[a]	0.17[c]	0.10[d]	0.24[a]	0.21[b]	0.11[d]	0.016	0.299	<0.001	0.003
12:0	3.03[c]	2.42[d]	1.76[e]	5.17[b]	5.56[a]	2.99[c]	0.251	<0.001	<0.001	<0.001
cis-9 12:1	0.081[b]	0.048[c]	0.033[c]	0.138[a]	0.137[a]	0.043[c]	0.0127	<0.001	<0.001	<0.001
trans-9 12:1	0.074[a]	0.053[b]	0.041[c]	0.058[b]	0.055[b]	0.025[d]	0.0055	0.033	<0.001	0.004
14:0	11.19[a]	10.25[b]	7.65[c]	11.33[a]	11.07[ab]	6.61[d]	0.416	0.923	<0.001	0.003
cis-9 14:1	0.925	0.850	0.750	0.195	0.167	0.086	0.0769	<0.001	<0.001	0.609
cis-12 14:1	0.016[b]	0.013[b]	0.011[b]	0.026[a]	0.027[a]	0.016[b]	0.0029	<0.001	<0.001	0.049
trans-6 14:1	0.001[d]	0.017[b]	0.004[c]	0.015[b]	0.025[a]	0.015[b]	0.0016	<0.001	<0.001	0.012
trans-9 14:1	0.022[a]	0.022[a]	0.018[b]	0.011[c]	0.016[b]	0.013[c]	0.0015	<0.001	0.004	0.018
16:0	30.1[a]	25.4[b]	19.2[d]	24.7[bc]	23.9[c]	16.2[e]	0.71	<0.001	<0.001	<0.001
10-O-16:0	0.006[c]	0.143[a]	0.010[c]	<0.001[c]	0.062[b]	<0.001[c]	0.0094	<0.001	<0.001	<0.001
cis-6 + 7 16:1	0.17	0.21	0.22	0.24	0.26	0.26	0.015	<0.001	<0.001	0.241
cis-9 16:1	1.23	1.66	1.18	0.56	1.01	0.36	0.100	<0.001	<0.001	0.273
cis-11 16:1	0.045[c]	0.073[a]	0.059[b]	0.025[d]	0.053[bc]	0.019[d]	0.0051	<0.001	<0.001	<0.001
cis-12 16:1	0.031[c]	0.075[a]	0.054[b]	0.032[c]	0.030[c]	0.034[c]	0.0039	<0.001	<0.001	<0.001
trans-6 + 7 + 8 16:1	0.037[c]	0.205[a]	0.204[a]	0.050[c]	0.132[b]	0.137[b]	0.0262	0.022	<0.001	0.028
trans-9 16:1[4]	0.45	0.94	0.59	0.51	1.05	0.59	0.035	0.029	<0.001	0.057
16:2n-4[5]	0.015	0.054	0.018	0.012	0.040	0.014	0.0036	0.001	<0.001	0.069
trans-10,*trans*-14 16:2	0.007	0.047	0.010	0.011	0.047	0.008	0.0045	0.756	<0.001	0.619
18:0	10.18[c]	3.17[e]	11.87[b]	7.74[d]	1.65[e]	14.70[a]	0.824	0.526	<0.001	<0.001
10-O-18:0	0.027[d]	0.751[a]	0.287[c]	0.032[d]	0.503[b]	0.212[c]	0.0531	0.007	<0.001	0.003
13-O-18:0	0.023	0.033	0.023	0.012	0.022	0.015	0.0024	<0.001	<0.001	0.416
Σ *cis* 18:1	17.77	9.17	23.93	17.01	6.31	21.94	0.949	0.009	<0.001	0.226
Σ *trans* 18:1	3.34[c]	14.51[a]	13.41[a]	2.63[c]	9.74[b]	9.30[b]	0.705	<0.001	<0.001	<0.001
Σ nonconjugated 18:2	2.94[c]	4.56[b]	4.58[b]	2.94[c]	3.26[c]	5.24[a]	0.275	0.277	<0.001	<0.001
Σ CLA	0.72[e]	2.83[b]	1.33[cd]	0.88[de]	4.45[a]	1.47[c]	0.230	<0.001	<0.001	<0.001
18:3n-6	0.036	0.023	0.034	0.035	0.030	0.038	0.0027	0.047	<0.001	0.111
18:3n-3	0.65	0.56	0.36	0.63	0.46	0.33	0.033	0.036	<0.001	0.187
Δ6,10,15 18:3[6]	0.033[bc]	0.036[ab]	0.035[ab]	0.034[ab]	0.039[a]	0.029[c]	0.0026	0.757	0.009	0.045
Δ9,12,15 18:3	0.015[c]	0.050[a]	0.003[d]	0.010[c]	0.032[b]	0.001[d]	0.0034	<0.001	<0.001	0.003
cis-9,*trans*-11,*trans*-15 18:3	0.021	0.123	0.062	0.024	0.114	0.058	0.0794	0.185	<0.001	0.697
24:0[7]	0.059[a]	0.047[b]	0.030[c]	0.032[c]	0.031[c]	0.027[d]	0.0027	<0.001	<0.001	<0.001
cis-11 24:1	ND[8]	0.027	ND	ND	0.025	ND	0.0024	0.432	—	—
cis-15 24:1	0.019	0.024	0.007	0.018	0.022	0.010	0.002	0.896	<0.001	0.201
26:0	0.050[a]	0.046[a]	0.027[c]	0.035[b]	0.046[a]	0.027[c]	0.0038	0.262	<0.001	<0.001
Ratio										
cis-9 10:1/10:0	0.096[a]	0.079[b]	0.070[c]	0.024[d]	0.020[de]	0.017[e]	0.0057	<0.001	<0.001	0.002
cis-9 12:1/12:0	0.027[a]	0.020[bc]	0.019[c]	0.026[a]	0.024[ab]	0.014[d]	0.0026	0.856	<0.001	<0.001
cis-9 14:1/14:0	0.083[b]	0.082[b]	0.096[a]	0.017[c]	0.015[c]	0.013[c]	0.0078	<0.001	0.129	0.008
cis-9 16:1/16:0	0.041[b]	0.066[a]	0.061[a]	0.023[c]	0.042[b]	0.022[c]	0.0045	<0.001	<0.001	<0.001
cis-9 18:1/18:0	1.73[cd]	2.61[b]	2.04[c]	2.14[c]	3.20[a]	1.40[d]	0.204	0.431	<0.001	<0.001
cis-9,*trans*-11 CLA/*trans*-11 18:1	0.41[b]	0.37[b]	0.43[b]	0.66[a]	0.60[a]	0.44[b]	0.043	<0.001	0.002	<0.001

Continued

Table 3 (Continued). Effect of dietary supplements of fish oil or sunflower oil and starch on milk FA composition in cows and goats[1]

	Cows			Goats				*P*-value[3]		
FA, g/100 g of FA	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
Apparent transfer,[9] %										
20:5n-3	—	3.32	—	—	3.06	—	0.519	0.620	—	—
22:5n-3	—	25.48	—	—	23.58	—	2.427	0.213	—	—
22:6n-3	—	4.75	—	—	4.61	—	0.684	0.839	—	—
Estimated milk fat melting point, °C	37.3	34.2	36.6	36.0	34.5	35.2	0.66	0.063	<0.001	0.117

[a–c]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; FO = basal diet supplemented with fish oil; SOS = basal diet containing sunflower oil and wheat starch.

[2]SED = standard error of the difference.

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

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

[5]Coelutes with *cis*-11 17:1.

[6]Coelutes with cyclo-18:0.

[7]Coelutes with *cis*-10,*cis*-13,*cis*-16 22:3.

[8]nd = not detected.

[9]Calculated as [grams of milk fat yield × (% FA in milk fat − % FA in control milk fat)/(DMI × % FA in the diet)] × 100.

Milk FA Composition

Even though species had a significant effect on the concentration of the major FA in milk, the extent of these differences on the control diet were quantitatively minor, except for higher ($P < 0.05$) 8:0, 10:0, and 12:0 and lower ($P < 0.05$) 4:0, 16:0, and 18:0 concentrations in milk from goats compared with cows (Table 3). Milk of goats also contained higher concentrations of 7- to 17-carbon odd-chain FA (except 15:0; Table 4) and certain MUFA (*cis*-9 12:1, *cis*-12 14:1, *trans*-6 14:1, and *cis*-6+7 16:1; Table 3). Furthermore, milk of goats contained lower proportions of *anteiso* 15:0, *anteiso* 17:0, several MUFA, including *cis*-9 14:1, *cis*-9 16:1, *trans*-9 14:1, *trans*-9 16:1 (Tables 3 and 4), *cis*- and *trans*-18:1 with double bonds at Δ13, Δ15, and Δ16 (Table 5), and *trans*,*trans* CLA isomers (Table 6). However, no differences were observed in the concentration of the most abundant 18:1 and 18:2 isomers, including *cis*-9 18:1, *trans*-10 18:1, *trans*-11 18:1, and *cis*-9,*trans*-11 CLA in milk from cows and goats fed the control diet (Tables 5 and 6).

Compared with the control, SOS decreased ($P < 0.05$) the concentration of 6- to 16-carbon even-chain saturated and unsaturated FA in milk of cows and goats (except for *trans*-6 14:1 and 16-carbon unsaturated FA; Table 3). Decreases in the concentration of 6:0, 8:0, and 10:0 to SOS were greater in cows, whereas the decrease in 14:0 relative to the control was higher in goats than cows. In cows, FO lowered ($P < 0.05$) the concentration of 6- to 16-carbon even-chain FA, but the decreases were much lower than to the SOS treatment (Table 3). In contrast, FO increased ($P < 0.001$) milk 12:0 concentration in goats. For both species, FO elevated ($P < 0.05$) milk *trans* 14:1, 16:1, and 16:2 concentrations (Table 3). The highest concentration of certain oxygenated FA (10-O-16:0 and 10-O-18:0) in milk fat was detected in milk of cows fed FO ($P < 0.01$). Treatment FO also elevated milk 10-O-18:0 concentration in goats, but to a lesser extent than in cows. Compared with the control, SOS increased milk 10-O-18:0 concentration in both species (Table 3).

Relative to the control, SOS decreased ($P < 0.05$) the concentration of most odd- and branched-chain FA in milk from both species, but elevated the proportions of 5:0 to 13:0 in milk from goats (Table 4). In contrast, FO generally enriched odd- and branched-chain FA concentration in milk from cows and goats. Specifically, FO lowered ($P < 0.001$) *iso* 14:0 and *cis*-10 19:1 in cow and goat milk, increased ($P < 0.05$) *anteiso* 13:0, *iso* 13:0, and *trans*-5 15:1 in milk from cows, 5:0 to 13:0 and *cis*-14 23:1 in milk from goats, and *anteiso* 15:0, *trans*-6 15:1, *iso* 18:0, 7-methyl-hexadecyl-6-enoate,

Table 4. Effect of dietary supplements of fish oil or sunflower oil and starch on milk odd- and branched-chain FA composition in cows and goats[1]

	Cows			Goats				*P*-value[3]		
FA, g/100 g of FA	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
5:0	0.035^{bc}	0.029^{c}	0.036^{bc}	0.032^{c}	0.044^{b}	0.062^{a}	0.0045	<0.001	<0.001	<0.001
7:0	0.026^{c}	0.019^{c}	0.021^{c}	0.042^{b}	0.070^{a}	0.079^{a}	0.0057	<0.001	<0.001	<0.001
9:0	0.023^{c}	0.017^{c}	0.018^{c}	0.075^{b}	0.131^{a}	0.130^{a}	0.0103	<0.001	<0.001	<0.001
11:0	0.038^{d}	0.027^{d}	0.033^{d}	0.116^{c}	0.200^{a}	0.161^{b}	0.0162	<0.001	0.005	<0.001
13:0	0.072^{c}	0.068^{c}	0.082^{c}	0.110^{b}	0.154^{a}	0.146^{a}	0.0115	<0.001	0.010	0.012
anteiso 13:0	0.015^{b}	0.017^{a}	0.014^{b}	0.013^{b}	0.014^{b}	0.009^{c}	0.0009	<0.001	<0.001	0.012
iso 13:0	0.037^{b}	0.046^{a}	0.039^{ab}	0.032^{b}	0.038^{b}	0.018^{c}	0.004	<0.001	<0.001	0.005
iso 14:0	0.107^{a}	0.070^{bc}	0.057^{c}	0.120^{a}	0.088^{b}	0.109^{a}	0.0087	<0.001	<0.001	0.003
15:0	0.99	1.03	0.81	1.06	1.16	0.81	0.054	0.047	<0.001	0.290
anteiso 15:0	0.49^{b}	0.52^{a}	0.43^{cd}	0.38^{d}	0.44^{c}	0.28^{e}	0.024	<0.001	<0.001	0.028
iso 15:0	0.25^{a}	0.26^{a}	0.17^{b}	0.27^{a}	0.27^{a}	0.14^{c}	0.013	0.889	<0.001	0.002
cis-9 15:1	0.016^{ab}	0.017^{a}	0.018^{a}	0.013^{b}	0.013^{b}	0.010^{c}	0.0015	<0.001	0.493	0.007
trans-5 15:1	0.077^{b}	0.099^{a}	0.036^{c}	0.085^{b}	0.086^{b}	0.036^{c}	0.0064	0.997	<0.001	0.021
trans-6 15:1	0.014	0.030	0.010	0.018	0.034	0.013	0.003	0.030	<0.001	0.861
iso 16:0[4]	0.26^{ab}	0.24^{b}	0.16^{c}	0.27^{ab}	0.24^{b}	0.30^{a}	0.023	0.006	0.218	<0.001
17:0[5]	0.70^{b}	0.70^{b}	0.54^{c}	0.81^{a}	0.81^{a}	0.52^{c}	0.024	<0.001	<0.001	<0.001
anteiso 17:0	0.42	0.45	0.38	0.35	0.34	0.32	0.023	<0.001	0.004	0.068
7-methyl-hexadecyl-6-enoate	0.026^{b}	0.089^{a}	0.002^{d}	0.014^{c}	0.083^{a}	0.011^{c}	0.0042	0.188	<0.001	<0.001
cis-8 17:1	0.041	0.054	0.041	0.049	0.044	0.032	0.0068	0.283	0.062	0.078
cis-9 17:1	0.20^{ab}	0.21^{a}	0.22^{a}	0.19^{ab}	0.17^{b}	0.13^{c}	0.018	<0.001	0.258	0.008
iso 18:0[6]	0.051^{c}	0.099^{a}	0.050^{c}	0.045^{c}	0.072^{b}	0.034^{d}	0.0051	<0.001	<0.001	0.02
2R,6R,10R,14-tetramethyl-15:0	0.024	0.032	0.014	0.020	0.021	0.013	0.0032	0.007	<0.001	0.079
cis-9 19:1	0.087	0.085	0.093	0.078	0.079	0.103	0.0070	0.730	<0.001	0.112
cis-10 19:1	0.055	0.037	0.055	0.050	0.040	0.049	0.0047	0.346	<0.001	0.318
21:0[7]	0.056^{c}	0.105^{a}	0.043^{d}	0.066^{c}	0.082^{b}	0.039^{d}	0.0052	0.082	<0.001	<0.001
cis-12 21:1	ND[8]	0.043	ND	ND	0.019	ND	0.0046	<0.001	—	—
23:0	0.027^{b}	0.036^{a}	0.017^{c}	0.015^{c}	0.023^{b}	0.013^{c}	0.002	<0.001	<0.001	0.008
cis-14 23:1	0.040^{c}	0.043^{bc}	0.026^{e}	0.045^{b}	0.061^{a}	0.032^{d}	0.002	<0.001	<0.001	<0.001

$^{a–e}$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; FO= basal diet supplemented with fish oil; SOS = basal diet containing sunflower oil and wheat starch.

[2]SED = standard error of the difference.

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

[4]Coelutes with 2S,6R,10R,14-tetramethyl-15:0.

[5]Coelutes with *cis*-13 + 15 16:1.

[6]Coelutes with *trans*-9,*trans*-13 16:2.

[7]Coelutes with *trans*-10,*trans*-16 20:2.

[8]nd = not detected.

Table 5. Effect of dietary supplements of fish oil or sunflower oil and starch on milk 18:1 isomer concentrations in cows and goats[1]

	Cows			Goats				*P*-value[3]		
FA, g/100 g of FA	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
cis-9 18:1[4]	16.75	7.56	21.99	16.14	5.00	20.13	0.945	0.015	<0.001	0.273
cis-11 18:1	0.64^{cd}	1.28^{a}	1.04^{b}	0.59^{d}	1.10^{b}	0.74^{c}	0.056	<0.001	<0.001	0.004
cis-12 18:1	0.231^{c}	0.144^{cd}	0.576^{b}	0.183^{cd}	0.093^{d}	0.858^{a}	0.0512	0.055	<0.001	<0.001
cis-13 18:1	0.093^{c}	0.128^{b}	0.215^{a}	0.065^{d}	0.093^{c}	0.139^{b}	0.0084	<0.001	<0.001	<0.001
cis-15 18:1[5]	0.16^{c}	0.26^{a}	0.20^{b}	0.14^{de}	0.12^{e}	0.15^{cd}	0.010	<0.001	<0.001	<0.001
cis-16 18:1	0.051	0.048	0.104	0.037	0.026	0.072	0.0058	<0.001	<0.001	0.064
trans-4 18:1	0.034^{cd}	0.038^{c}	0.101^{a}	0.024^{d}	0.038^{c}	0.072^{b}	0.0059	<0.001	<0.001	0.007
trans-5 18:1	0.028^{de}	0.043^{c}	0.108^{a}	0.021^{e}	0.038^{cd}	0.077^{b}	0.0064	<0.001	<0.001	0.008
trans-6 + 7 + 8 18:1	0.25^{d}	0.45^{c}	1.10^{a}	0.19^{d}	0.29^{d}	0.70^{b}	0.059	<0.001	<0.001	0.0002
trans-9 18:1	0.22^{d}	0.58^{b}	0.70^{a}	0.22^{d}	0.42^{c}	0.54^{b}	0.029	<0.001	<0.001	<0.001
trans-10 18:1	0.42^{c}	4.10^{b}	6.34^{a}	0.29^{c}	0.69^{c}	3.17^{b}	0.806	<0.001	<0.001	0.005
trans-11 18:1	1.25	7.17	2.15	1.15	7.00	2.51	0.497	0.928	<0.001	0.666
trans-12 18:1	0.32^{d}	0.96^{a}	1.05^{a}	0.25^{d}	0.62^{c}	0.83^{b}	0.045	<0.001	<0.001	<0.001
trans-13 + 14 18:1	0.51	0.99	1.28	0.29	0.56	0.93	0.072	<0.001	<0.001	0.123
trans-15 18:1	0.56	0.52	0.97	0.46	0.30	0.85	0.046	<0.001	<0.001	0.118
trans-16 18:1[6]	0.31	0.18	0.57	0.21	0.08	0.47	0.025	<0.001	<0.001	0.893

[a–d]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; FO = basal diet supplemented with fish oil; SOS = basal diet containing sunflower oil and wheat starch.

[2]SED = standard error of the difference.

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

[4]Contains *cis*-10 18:1 as a minor component.

[5]Coelutes with *trans*-10,*trans*-14 18:2 and 19:0.

[6]Coelutes with *cis*-14 18:1, *trans*-5,*trans*-10 18:2, and *trans*-5,*trans*-11 18:2.

tetramethyl branched-chain FA, 21:0, and 23:0 concentrations in milk from both species (Table 4).

Across diets and species, the abundance of 18-carbon FA in milk varied substantially. Relative to the control, FO resulted in a substantial decrease ($P < 0.001$) in milk 18:0 concentration (mean 74% for both species; Table 3 and Figure 2), whereas the proportionate change in *cis*-9 18:1 concentration was greater in goats compared with cows (−69 vs. −55%; Table 5 and Figure 2). In contrast, SOS increased 18:0 and *cis*-9 18:1 concentration ($P < 0.001$), with the relative enrichment of 18:0 being substantially greater in milk from goats than cows (mean relative increases 90 and 17%, respectively).

In general, both FO and SOS increased the concentration of *cis*- and *trans*-18:1 isomers (Table 5). The most abundant *trans* FA in milk of cows and goats fed the control diet was *trans*-11 18:1. Relative to the control, FO increased ($P < 0.001$) milk *trans*-18:1 concentration ~5-fold in both species (Figure 2). However, changes in milk *trans*-10 18:1 concentration to dietary treatments differed between cows and goats. In goats, FO had no effect on milk *trans*-10 18:1 concentration, but caused an 8.8-fold increase in cows (Figure 2). For both species, SOS increased ($P < 0.01$) *trans*-10 18:1 concentrations, but the abundance was 2-fold higher in milk from cows than goats (Table 5). Similarly, SOS resulted in a greater increase in the concentration of other *trans*-18:1 isomers in milk compared with FO in both species, with the magnitude of increase being higher in cows compared with goats (Table 5).

Relative to the control, FO and SOS increased the concentration of most 18:2 isomers in milk for both species (Table 6), but FO decreased ($P < 0.05$) 18:2n-6 in goats, and FO and SOS lowered milk *trans*-11,*cis*-13 CLA and *trans*-11,*trans*-13 CLA in cows and goats. Compared with the control and SOS, FO elevated ($P < 0.05$) the proportions of 18:2 isomers containing a *cis*-15 double bond, *trans*-11,*trans*-15 18:2, and *trans*-7,*trans*-9 CLA in both species, as well as *trans*-9,*trans*-12 18:2 in cows, with the enrichment being higher in milk from cows than goats. Responses to SOS in both species were characterized by higher ($P < 0.05$) proportions of 18:2n-6, *cis*-9,*trans*-13 18:2, *cis*-9,*trans*-14 18:2, *trans*-9,*trans*-13 18:2, *trans*-10,*trans*-14 18:2, *trans*-7,*cis*-9 CLA, *trans*-10,*cis*-12 CLA, *trans*-8,*trans*-10 CLA, and *trans*-10,*trans*-12 CLA compared with FO and the control. For both species, FO resulted in the highest ($P < 0.001$) *cis*-9,*trans*-11 CLA enrichment of 4.1 and 2.6% of total FA in milk from goats and cows, respectively, whereas the SOS treatment only increased *cis*-9,*trans*-11 CLA concentrations in goats (1.1% of total FA; Table 6 and Figure 2). Compared with the control, milk *trans*-9,*cis*-11 CLA concentration was increased ($P = 0.028$) 5.7-fold to FO in goats and 4.6-fold to FO and SOS in cows (Figure 2). Concentrations of

Table 6. Effect of dietary supplements of fish oil or sunflower oil and starch on milk 18:2 isomer concentrations in cows and goats[1]

	Cows			Goats				*P*-value[3]		
FA, mg/100 g of FA	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
cis-9,*cis*-12 18:2[4]	2,276[c]	1,963[c]	3,300[b]	2,296[c]	1,484[d]	4,145[a]	220.9	0.395	<0.001	<0.001
cis-11,*cis*-14 18:2	ND[5]	59.4	ND	ND	51.8	ND	6.61	0.259	—	—
cis-11,*cis*-15 18:2[6]	5.49	36.03	10.81	0.99	29.45	3.75	2.39	<0.001	<0.001	0.694
cis-12,*cis*-15 18:2[7]	21.4[b]	40.4[a]	17.4[b]	17.4[b]	21.4[b]	18.5[b]	2.32	<0.001	<0.001	<0.001
cis-9,*trans*-12 18:2	33.0	65.7	76.0	48.7	78.9	90.1	6.12	0.001	<0.001	0.955
cis-9,*trans*-13 18:2[8]	180[d]	327[b]	400[a]	190[d]	246[c]	373[ab]	24.6	0.084	<0.001	0.012
cis-9,*trans*-14 18:2	88.1	84.2	163.0	90.2	56.1	152.3	10.95	0.107	<0.001	0.112
cis-9,*trans*-15 18:2[9]	33.0[c]	66.0[b]	70.2[b]	30.2[c]	36.2[c]	94.1[a]	6.33	0.445	<0.001	<0.001
trans-5,*cis*-9 18:2[10]	63.4[bc]	60.3[bcd]	176.8[a]	46.2[cd]	42.0[d]	76.3[b]	10.38	<0.001	<0.001	<0.001
trans-9,*cis*-12 18:2[11]	32.9[d]	115.2[a]	68.1[bc]	31.8[d]	73.9[b]	63.4[c]	5.15	<0.001	<0.001	<0.001
trans-11,*cis*-15 18:2[12]	110.2[c]	1,320.7[a]	86.4[c]	96.5[c]	879.2[b]	58.3[c]	80.16	0.003	<0.001	<0.001
trans-12,*cis*-15 18:2[13]	21.5[c]	103.9[a]	19.2[c]	16.7[c]	46.9[b]	20.8[c]	4.11	<0.001	<0.001	<0.001
trans-9,*trans*-12 18:2[14]	23.0[b]	119.6[a]	43.1[b]	28.4[b]	46.5[b]	37.4[b]	12.71	0.005	<0.001	<0.001
trans-9,*trans*-13 18:2[15]	38.3[d]	65.5[c]	127.3[a]	43.2[d]	65.5[c]	102.3[b]	8.00	0.177	<0.001	0.019
trans-11,*trans*-15 18:2	37.9[c]	172.3[a]	34.4[cd]	23.8[cd]	128.8[b]	21.4[d]	7.93	<0.001	<0.001	0.009
cis-9,*cis*-11 CLA	2.2	2.1	1.4	2.1	3.8	1.9	0.64	0.044	0.012	0.122
cis-9,*trans*-11 CLA	585[d]	2,555[b]	882[cd]	750[d]	4,145[a]	1,144[c]	228.0	<0.001	<0.001	<0.001
cis-11,*trans*-13 CLA	1.73[bc]	2.24[ab]	2.79[a]	1.25[c]	1.62[bc]	1.19[c]	0.33	<0.001	0.062	0.030
cis-12, *trans*-14 CLA	1.09[b]	1.68[ab]	2.48[a]	1.69[ab]	1.08[b]	1.17[b]	0.41	0.089	0.197	0.005
trans-6,*cis*-8 CLA	4.06[c]	4.65[c]	25.80[a]	5.18[c]	14.30[bc]	15.13[b]	5.27	0.992	<0.001	0.019
trans-7,*cis*-9 CLA	34.0[d]	81.4[b]	153.9[a]	38.4[cd]	56.2[c]	98.8[b]	10.80	<0.001	<0.001	<0.001
trans-8,*cis*-10 CLA	6.38	14.13	11.98	9.17	21.27	12.71	2.63	0.041	<0.001	0.174
trans-9,*cis*-11 CLA	21.8[c]	99.5[ab]	99.9[ab]	25.9[c]	148.2[a]	53.3[bc]	26.09	0.888	<0.001	0.028
trans-10,*cis*-12 CLA	3.51	5.00	59.73[a]	2.94	1.51	64.22	10.99	0.982	<0.001	0.859
trans-11,*cis*-13 CLA	9.81	7.52	4.64	12.08	7.31	2.48	1.59	0.968	<0.001	0.127
trans-12,*cis*-14 CLA	4.32	4.79	3.41	2.97	2.45	2.59	0.91	0.004	0.487	0.446
trans-7,*trans*-9 CLA	6.27	10.18	5.96	3.49	6.34	3.36	1.39	<0.001	<0.001	0.771
trans-8,*trans*-10 CLA	2.73	5.57	16.08	2.03	5.89	12.87	1.06	0.066	<0.001	0.051
trans-9,*trans*-11 CLA	15.1	14.1	19.0	12.6	16.3	23.3	2.77	0.462	<0.001	0.166
trans-10,*trans*-12 CLA	4.17	9.53	30.59	3.01	9.92	29.88	4.31	0.846	<0.001	0.964
trans-11,*trans*-13 CLA	8.29	4.26	6.03	5.65	3.21	5.22	0.81	0.007	<0.001	0.186
trans-12,*trans*-14 CLA	4.40	4.10	2.83	1.94	3.15	1.78	0.597	<0.001	0.006	0.112

[a–e]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; FO = basal diet supplemented with fish oil; SOS = basal diet containing sunflower oil and wheat starch.

[2]SED = standard error of the difference.

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

[4]Coelutes with *cis*-9,*cis*-14 18:2 and *cis*-9,*cis*-15 18:2.

[5]nd = not detected.

[6]Coelutes with *cis*-10,*cis*-15 18:2.

[7]Coelutes with *cis*-12,*cis*-16 18:2 and *cis*-13,*cis*-16 18:2.

[8]Coelutes with 11-cyclohexyl-11:0, *cis*-10,*trans*-14 18:2, *trans*-10,*trans*-13 18:2, and *trans*-11,*trans*-14 18:2.

[9]Coelutes with *cis*-8,*cis*-12 18:2, *trans*-8,*cis*-15 18:2, and *trans*-10,*cis*-14 18:2.

[10]Coelutes with *trans*-6,*cis*-10 18:2.

[11]Coelutes with *cis*-12,*trans*-16 18:2 and *trans*-11,*cis*-16 18:2.

[12]Coelutes with *trans*-10,*cis*-15 18:2.

[13]Coelutes with *cis*-11 19:1 and *cis*-11,*cis*-16 18:2.

[14]Coelutes with *trans*-10,*trans*-15 18:2.

[15]Coelutes with *cis*-5,*trans*-9 18:2.

trans-9,*trans*-11 CLA were similar in milk from both species and increased ($P = 0.001$) in response to the SOS treatment.

Both FO and SOS decreased ($P < 0.001$) milk 18:3n-3 concentration, with the lowest proportions detected in milk on the SOS treatment for both species (Table 3). Compared with the control, FO elevated ($P < 0.01$) milk Δ9,12,15 18:3 concentration, with the increase being higher in cows than goats.

For both species, FO increased ($P < 0.05$) the concentrations of all identified 20- and 22-carbon unsaturated FA in milk (Table 7). Several 20- and 22-carbon isomers were only present in milk on the FO treatment. In general, the increase ($P < 0.05$) in 20- and 22-carbon FA to FO were greater in milk from cows than goats, except for the larger proportionate increases ($P < 0.05$) of *trans*-6,*cis*-11,*cis*-14,*cis*-17 20:4, 22:5n-3, and 22:6n-3 in milk from goats, and a similar enrichment ($P < 0.001$) of 20:5n-3 in both species. However, the apparent transfer of n-3 long-chain PUFA from fish oil into milk did not differ ($P > 0.10$) between species, averaging 3.2, 24.5, and 4.7% for 20:5n-3, 22:5n-3, and 22:6n-3, respectively (Table 3). Relative to the control, SOS increased ($P < 0.001$) 22:4n-6 concentrations in milk from cows and goats, resulted in a higher abundance of 20:4n-6 in goat milk and 20:3n-6 in cow milk, and lowered the proportion of 20:2n-6 in cow milk. For both species, SOS lowered ($P < 0.001$) milk 22:5n-3 concentration compared with the control and FO.

On the control, *cis*-9 10:1/10:0, *cis*-9 14:1/14:0, and *cis*-9 16:1/16:0 concentration ratios were higher ($P < 0.001$) in milk from cows than goats, whereas the reverse was true for the *cis*-9,*trans*-11 CLA-to-*trans*-11 18:1 ratio (Table 3). In both species, FO increased ($P < 0.001$) the ratio of *cis*-9 16:1/16:0 and *cis*-9 18:1/18:0, whereas SOS decreased ($P < 0.001$) the ratio of *cis*-9 10:1/10:0 and *cis*-9 12:1/12:0. However, the decrease in *cis*-9 10:1/10:0 and *cis*-9 12:1/12:0 ratios to FO and the increase in the ratio of *cis*-9 14:1/14:0 and *cis*-9 16:1/16:0 to SOS were only observed in milk from cows. Furthermore, a decrease ($P < 0.001$) in the ratio of *cis*-9 18:1/18:0 to SOS was exclusive to milk from goats.

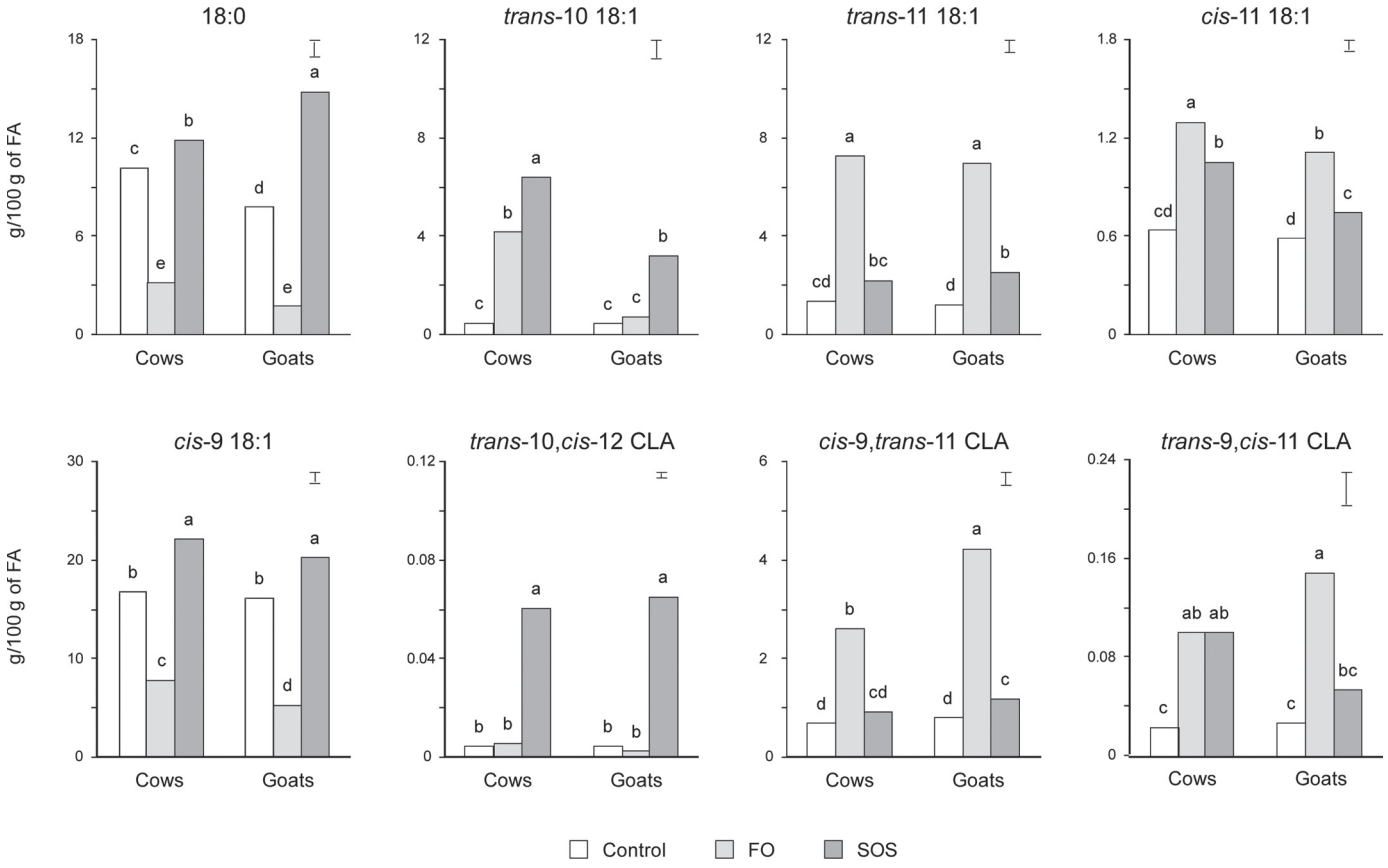

Figure 2. Milk 18:0, *cis*-11 18:1, *trans*-11 18:1, *trans*-10 18:1, *cis*-9 18:1, *trans*-9,*cis*-11 CLA, *cis*-9,*trans*-11 CLA, and *trans*-10,*cis*-12 CLA concentrations in cows and goats fed a basal diet containing no additional lipid (control), or supplemented with fish oil (FO) or sunflower oil and additional starch from flattened wheat (SOS). Within each FA, values not sharing a common letter (a-e) differ at $P < 0.05$. Vertical bars indicate standard error of the difference.

Table 7. Effect of dietary supplements of fish oil or sunflower oil and starch on milk 20- and 22-carbon FA concentrations in cows and goats[1]

	Cows			Goats				*P*-value[3]		
FA, g/100 g of FA	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
20:0	0.168^{bc}	0.175^{abc}	0.124^{d}	0.191^{a}	0.166^{c}	0.183^{ab}	0.0097	0.005	<0.001	<0.001
cis-9 20:1	0.143^{b}	0.197^{a}	0.114^{c}	0.036^{e}	0.064^{d}	0.031^{e}	0.0076	<0.001	<0.001	<0.001
cis-11 20:1	0.045^{cd}	0.231^{a}	0.063^{c}	0.031^{d}	0.171^{b}	0.046^{cd}	0.0108	<0.001	<0.001	0.006
cis-13 20:1	0.005^{d}	0.068^{a}	0.013^{c}	0.007^{d}	0.051^{b}	0.006^{d}	0.0024	<0.001	<0.001	0.032
trans-11 20:1	0.003	0.054	0.005	0.004	0.041	0.006	0.0063	0.297	0.001	0.217
trans-12 20:1[4]	0.016^{e}	0.080^{a}	0.037^{c}	0.030^{cd}	0.046^{b}	0.029^{d}	0.0033	0.002	<0.001	<0.001
trans-13 20:1	0.007^{d}	0.092^{a}	0.025^{c}	0.013^{d}	0.060^{b}	0.015^{cd}	0.0057	<0.001	<0.001	<0.001
20:2n-6	0.061^{b}	0.097^{a}	0.054^{c}	0.033^{d}	0.062^{b}	0.030^{d}	0.0032	<0.001	<0.001	0.014
cis-10,*trans*-15 20:2	0.015^{c}	0.162^{a}	0.005^{cd}	0.015^{c}	0.134^{b}	0.004^{d}	0.0056	0.005	<0.001	<0.001
trans-9,*trans*-15 20:2	ND[5]	0.095	ND	ND	0.047	ND	0.0097	<0.001	—	—
trans-11,*cis*-16 20:2	ND	0.072	ND	ND	0.051	ND	0.0096	0.039	—	—
trans-13,*cis*-17 20:2[6]	0.002^{c}	0.170^{a}	0.022^{c}	0.008^{c}	0.060^{b}	0.012^{c}	0.0116	<0.001	<0.001	<0.001
20:2[7]	ND	0.037	ND	ND	0.036	ND	0.0081	0.941	—	—
20:3n-6[8]	0.072	0.125	0.085	0.028	0.074	0.028	0.0050	<0.001	<0.001	0.091
20:3n-3	0.027^{c}	0.195^{a}	0.012^{c}	0.015^{c}	0.125^{b}	0.010^{c}	0.0177	0.011	<0.001	0.015
Δ8,11,15 20:3[9]	ND	0.085	ND	ND	0.054	ND	0.0091	0.003	—	—
Δ8,12,17 20:3[10]	ND	0.042	ND	ND	0.020	ND	0.0069	0.004	—	—
Δ10,14,17 20:3	ND	0.039	ND	ND	0.017	ND	0.0053	<0.001	—	—
Δ11,14,17 20:3	ND	0.039	ND	ND	0.017	ND	0.0033	<0.001	—	—
trans-10,*trans*-14,*cis*-17 20:3[11]	ND	0.141	ND	ND	0.070	ND	0.0123	<0.001	—	—
trans-6,*trans*-12,*trans*-17 20:3[12]	ND	0.037	ND	ND	0.029	ND	0.0083	0.321	—	—
trans-9,*trans*-14,*trans*-17 20:3	ND	0.027	ND	ND	0.024	ND	0.0023	0.125	—	—
20:4n-6	0.10^{d}	0.15^{abc}	0.10^{d}	0.13^{c}	0.17^{a}	0.15^{b}	0.011	<0.001	<0.001	0.014
20:4n-3	0.053^{c}	0.572^{a}	0.031^{c}	0.014^{c}	0.406^{b}	0.009^{c}	0.0309	<0.001	<0.001	0.003
Δ8,11,14,17 20:4	ND	0.041	ND	ND	0.042	ND	0.0055	0.992	—	—
cis-7,*cis*-11,*cis*-14,*cis*-17 20:4	ND	0.060	ND	ND	0.046	ND	0.0070	0.063	—	—
trans-6,*cis*-11,*cis*-14,*cis*-17 20:4	ND	0.13	ND	ND	0.17	ND	0.014	0.003	—	—
20:5n-3	0.104	0.472	0.048	0.103	0.569	0.047	0.0439	0.258	<0.001	0.184
22:0[13]	0.088^{a}	0.087^{a}	0.058^{b}	0.069^{b}	0.062^{b}	0.081^{a}	0.0056	0.058	0.051	<0.001
cis-9 22:1[14]	ND	0.059^{a}	ND	ND	0.027	ND	0.0050	<0.001	—	—
cis-11 22:1	ND	0.077	ND	ND	0.065	ND	0.0063	0.082	—	—
cis-13 22:1	0.015	0.068	0.02	0.020	0.047	0.013	0.0058	0.134	<0.001	0.063
cis-15 22:1	ND	0.016	ND	ND	0.018	ND	0.0033	0.537	—	—
trans-11 22:1[15]	ND	0.031	ND	ND	0.020	ND	0.0036	0.006	—	—
trans-12 22:1	ND	0.028	ND	ND	0.018	ND	0.0012	<0.001	—	—
22:2n-6	0.001	0.019	0.004	0.001	0.015	<0.001	0.0017	0.011	<0.001	0.202
22:2n-3	ND	0.018	ND	ND	0.019	ND	0.0016	0.539	—	—
Δ10,13,17 22:3[16]	ND	0.018	ND	ND	0.019	ND	0.0016	0.462	—	—
Δ10,14,19 22:3	ND	0.042	ND	ND	0.041	ND	0.0046	0.829	—	—
22:4n-6	0.017	0.024	0.022	0.015	0.021	0.019	0.0019	0.044	<0.001	0.896
22:4n-3	ND	0.047	ND	ND	0.051	ND	0.0055	0.492	—	—
22:4 Δ10,13,16,19	ND	0.028	ND	ND	0.009	ND	0.0022	0.020	—	—
22:5n-6	0.021^{c}	0.215^{a}	0.010^{c}	0.009^{c}	0.112^{b}	0.008^{c}	0.0119	<0.001	<0.001	<0.001
22:5n-3	0.155^{cd}	0.442^{b}	0.097^{e}	0.169^{c}	0.554^{a}	0.096^{de}	0.0291	0.054	<0.001	0.006

Continued

Table 7 (Continued). Effect of dietary supplements of fish oil or sunflower oil and starch on milk 20- and 22-carbon FA concentrations in cows and goats[1]

	Cows			Goats				*P*-value[3]		
FA, g/100 g of FA	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
22:6n-3	0.053[cd]	0.274[b]	0.022[d]	0.083[c]	0.376[a]	0.047[cd]	0.0227	0.001	<0.001	0.031

[a–d]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; FO = basal diet supplemented with fish oil; SOS = basal diet containing sunflower oil and wheat starch.
[2]SED = standard error of the difference.
[3]Probability of significant effects due to species (Sp), experimental diet (D), and their interaction (Sp × D).
[4]Coelutes with 18:3n-4.
[5]nd = not detected.
[6]Contains 21:1 of indeterminate double bond position as minor component.
[7]Double bond position and geometry indeterminate.
[8]Coelutes with *cis*-9 21:1.
[9]Coelutes with *cis*-9,*trans*-11,*cis*-15 18:3.
[10]Coelutes with Δ8,13,17 20:3.
[11]Coelutes with *trans*-9,*trans*-11,*cis*-15 18:3 (tentative assignment of double bond geometry), *cis*-11,*cis*-14,*trans*-17 20:3, and Δ5,8,11,15 20:4.
[12]Coelutes with *cis*-14,*cis*-17 20:2.
[13]Coelutes with Δ10,14,17 20:3.
[14]Coelutes with Δ11,14,17 20:3.
[15]Coelutes with Δ11,14,17 20:3.
[16]Coelutes with 23:1 of indeterminate double bond position.

Plasma Metabolite and Hormone Concentrations

Plasma glucose, NEFA, insulin, and leptin concentrations were similar ($P > 0.10$) among species, but circulating concentrations of BHBA and IGF-I were higher ($P < 0.01$) in cows than goats (Table 8). Relative to the control, FO decreased ($P < 0.001$) plasma insulin concentrations in both species. For both species, SOS decreased ($P = 0.001$) BHBA and increased ($P = 0.001$) NEFA concentrations, but elevated ($P = 0.015$) plasma glucose concentrations in cows.

DISCUSSION

A more complete understanding of nutritional regulation of milk fat secretion would contribute to the development of feeding and management practices for altering milk FA composition and optimizing milk fat production (Harvatine et al., 2009; Shingfield et al., 2010). Our study compared the responses to diets formulated to induce changes in milk fat synthesis in goats and cows and differences in lipid metabolism to provide further insight into the mechanisms regulating mammary lipogenesis in ruminants.

Species Specificities on the Control Diet

Direct comparison of milk fat composition for cows and goats fed the same control diet provided clear evidence of interspecies differences in milk fat secretion and FA composition and, by inference, formation of specific BH intermediates in the rumen. First, the much lower *cis*-9 10:1/10:0, *cis*-9 14:1/14:0, and *cis*-9 16:1/16:0 concentration ratios in milk of goats than cows (Table 3) would tend to implicate a more extensive Δ^9-desaturation of FA in the bovine than caprine mammary gland. These findings are in agreement with indirect comparisons of milk FA composition from cows and goats fed similar diets (Chilliard et al., 2007; Bernard et al., 2009; Roy et al., 2006). A higher *cis*-9,*trans*-11 CLA/*trans*-11 18:1 concentration ratio for milk from goats than cows may reflect a greater affinity of the Δ^9-desaturase enzyme for *trans*-11 18:1 in the caprine than bovine mammary gland. Second, milk from goats contained higher concentrations of 8:0, 10:0, and 12:0 than cows, suggesting a proportionately higher synthesis de novo of these FA in goats than cows (Chilliard et al., 2003). Third, differences in the concentrations of BH intermediates in milk also point toward possible differences in ruminal lipolysis and BH pathways between ruminant species. A higher abundance of 18:1 isomers with double bond positions at Δ13 to Δ16 and *trans*,*trans* Δ7,9 CLA, Δ11,13 CLA, and Δ12,14 CLA isomers in milk of cows compared with goats could be considered as evidence

Table 8. Effect of dietary supplements of fish oil or sunflower oil and starch on plasma metabolite and hormone concentrations in cows and goats[1]

	Cows			Goats				*P*-value[3]		
Item	Control	FO	SOS	Control	FO	SOS	SED[2]	Sp	D	Sp × D
Glucose, g/L	0.653[b]	0.652[b]	0.709[a]	0.642[b]	0.622[b]	0.625[b]	0.0242	0.046	0.064	0.015
NEFA, m*M*	0.145	0.113	0.245	0.134	0.108	0.222	0.0480	0.634	0.001	0.961
BHBA, m*M*	0.555	0.474	0.305	0.347	0.307	0.117	0.0590	<0.001	<0.001	0.833
Insulin, μIU[4]/mL	17.4	13.8	17.5	20.3	14.6	19.5	1.87	0.174	<0.001	0.664
IGF-I, ng/mL	129.3	156.2	126.0	97.7	99.5	93.6	14.06	0.002	0.036	0.150
Leptin, ng/mL	2.13	2.31	2.39	2.11	2.20	2.17	0.502	0.813	0.254	0.588

[a–d]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; FO = basal diet supplemented with fish oil; SOS = basal diet containing sunflower oil and wheat starch.

[2]SED = standard error of the difference.

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

[4]One μIU/mL is equivalent to 6.945 pmol/L of insulin.

of greater diversity of FA metabolic pathways in the rumen, or at least a higher propensity for diet-induced alterations in ruminal BH in the bovine than caprine.

Response to a Starch-Rich Diet plus Sunflower Oil

Supplements of SOS lowered DMI in cows, but not in goats, without altering milk yield in either species, responses that are consistent with earlier reports in goats (Bernard et al., 2009) and cows (Roy et al., 2006). Furthermore, comparisons of responses to the SOS treatment serve to highlight differences in the milk fat secretion to changes in diet composition between ruminant species. In cows, SOS caused a decrease in milk fat content and yield, but not in goats. Earlier reports have demonstrated that high-starch diets containing plant oil induce MFD in cows (Griinari et al., 1998; Peterson et al., 2003; Roy et al., 2006) but have no adverse effects on milk fat synthesis in goats (Chilliard et al., 2007; Bernard et al., 2009; Martínez Marín et al., 2012). These differences may, at least in part, be attributed to interspecies differences in the sensitivity of the mammary gland to increases in the supply of BH intermediates known to exhibit antilipogenic effects, which may explain MFD on diets containing relatively high amounts of starch and PUFA in cows but not in goats (Chilliard et al., 2007; Shingfield et al., 2010).

Studies involving postruminal infusions of FA have established *trans*-10,*cis*-12 CLA as the only BH intermediate shown unequivocally to inhibit milk fat synthesis (Shingfield et al., 2010). Compared with the control, SOS increased 17- and 22-fold *trans*-10,*cis*-12 CLA concentration in milk from cows and goats, respectively. Postruminal administration of high doses of *trans*-10,*cis*-12 CLA leads to a disproportionate decrease in the secretion of FA synthesized de novo compared with longer-chain FA taken up from the blood (Bauman and Griinari, 2003; Shingfield and Griinari, 2007), changes that also characterized MFD in cows fed the SOS treatment (Figure 1). In goats, an increase in the output of long-chain FA on the SOS treatment compensated for the decrease in short- and medium-chain FA, allowing milk fat secretion to be maintained (Figure 1). Based on the relationship between percentage decreases in milk fat yield with milk fat *trans*-10,*cis*-12 CLA concentrations in cows offered supplements of calcium salts or lipid encapsulated sources of *trans*-10,*cis*-12 CLA (Shingfield et al., 2010), the enrichment of *trans*-10,*cis*-12 CLA in milk would only account for up to 66% of the overall decrease in milk fat synthesis on the SOS treatment in cows. Use of the same relationship reported in goats (Shingfield et al., 2009a, 2010) indicated that the increase in milk *trans*-10,*cis*-12 CLA concentration on the SOS treatment in goats would be expected to be accompanied by a 6% decrease in milk fat yield, which is similar to the observed nonsignificant decrease (−4%: Table 2). Indirect comparisons of milk fat yield responses to various *trans*-10,*cis*-12 CLA-containing supplements (e.g., de Veth et al., 2005; de Andrade and Schmidely, 2006; Lock et al., 2008; Shingfield et al., 2009a) also support the hypothesis that the sensitivity of mammary lipogenesis to the inhibitory effects of *trans*-10,*cis*-12 CLA is several-fold lower in goats than cows (Shingfield et al., 2009a, 2010).

Ruminal synthesis was not determined, but considerations of the changes in milk fat composition suggest that increases in *trans*-10,*cis*-12 CLA at the mammary gland do not, in isolation, fully explain MFD to SOS in cows. Other BH intermediates with potential antilipogenic effects, including *cis*-10,*trans*-12 CLA, *trans*-9,*cis*-11 CLA, and *trans*-10 18:1 have also been implicated in diet-induced MFD (Shingfield and Griinari, 2007). Concentrations of *trans*-9,*cis*-11 CLA are known

to be increased in milk from cows fed diets containing high amounts of 18:2n-6 or concentrates (Roy et al., 2006; Chilliard et al., 2007), but not (Bernard et al., 2009) or to a much lesser extent (Martínez Marín et al., 2012) in goats, observations consistent with the responses to SOS in the present study (Table 6 and Figure 2). In both species, SOS resulted in *trans*-10 18:1 replacing *trans*-11 18:1 as the major *trans* FA in milk. Even though *trans*-10 18:1 has been considered as a candidate milk fat inhibitor, reports on the effects on lipogenesis in cows are equivocal (Lock et al., 2007; Kadegowda et al., 2009; Shingfield et al., 2009b) and there are no reports in goats. Irrespective of the efficacy of *trans*-10 18:1 in regulating mammary lipogenesis, an increase in *trans*-10 18:1 concentration has been suggested as a biomarker of altered ruminal BH pathways associated with MFD in cows, although this and earlier reports indicate it is not a useful diagnostic in goats (Shingfield et al., 2010; Martínez Marín et al., 2012; Bernard et al., 2015). Nevertheless, the shift from *trans*-11 18:1 to *trans*-10 18:1 to SOS was far more pronounced in cows, and *trans*-10 18:1 concentration was more than 2-fold higher in milk from cows than goats (Figure 2). This observation supports previous studies suggesting that, over a range of diets, ruminal BH in cows is much more susceptible to the *trans*-10 shift than goats (Roy et al., 2006; Bernard et al., 2009; Toral et al., 2014). Greater increases in other *trans*-18:1 isomers and lower increases in 18:0 concentrations in milk to SOS in cows compared with goats could also be considered as evidence that the caprine is less sensitive to diet-induced alterations in ruminal BH pathways than the bovine (Chilliard et al., 2007, 2014; Shingfield et al., 2010). However, the increase in milk *trans*-10,*cis*-12 CLA, *trans*-10,*trans*-12 CLA, and 10-O-18:0 concentration to SOS in cows and goats also highlights certain similarities among both ruminant species. Furthermore, SOS enriched *trans*-9,*trans*-11 CLA in milk from goats but not cows, a finding that is in agreement with indirect species comparisons (Roy et al., 2006; Bernard et al., 2009). Differences in the appearance and the abundance of intermediates in milk from goats and cows on the SOS treatment highlight substantial variability in the adaptations of BH to changes in diet composition and FA supply between ruminant species that merits further investigation.

Even though differences in circulating metabolites may also contribute to between-species variations in milk fat responses on the SOS treatment, this was not substantiated in the present study. Plasma BHBA concentrations were decreased to a similar extent on the SOS treatment in both species, whereas the absolute concentration of BHBA was higher in cows. Furthermore, decreases in circulating BHBA concentrations on the SOS treatment were not fully explained by decreases in DMI (Table 2 and Figure 1) or accompanied by changes in milk fat yield in goats.

Several BH intermediates have been reported to inhibit Δ^9-desaturase activity, including *trans*-9,*trans*-11 CLA, *trans*-10,*trans*-12 CLA, and *trans*-10,*cis*-12 CLA (Bernard et al., 2013). Milk fat concentrations of these FA were increased on the SOS treatment in both goats and cows, other than *trans*-9,*trans*-11 CLA enrichment that was confined to goats. Similarly, the SOS treatment resulted in a marginal or significant decrease in the major 14- to 18-carbon FA concentration ratios for Δ^9-desaturase in milk from goats, whereas no changes or an increase was observed in cows, consistent with indirect comparisons of diet-induced changes in milk FA composition in these species (Chilliard et al., 2007; Bernard et al., 2013). It is possible that such differences are related to variation in the availability of *trans*-9,*trans*-11 CLA at the mammary gland, or interspecies differences in the sensitivity of mammary Δ^9-desaturase to the inhibitory effects of PUFA.

Response to FO Supplements

Supplements of FO lowered DMI by 14% in cows consistent with earlier reports (e.g., Loor et al., 2005a; Gama et al., 2008), whereas FO had no adverse effects on DMI in goats (Table 2), as demonstrated previously (Toral et al., 2014). For both species, the FO treatment resulted in the appearance of numerous 20- and 22- carbon FA not contained in the FO supplement, such as *trans*-9,*trans*-15 20:2, Δ10,14,17 20:3, *trans*-10,*trans*-14,*cis*-17 20:3, *trans*-9,*trans*-14,*trans*-17 20:3, Δ10,13,17 22:3, Δ10,14,19 22:3, and Δ10,13,16,19 22:4. These findings confirm earlier reports in lactating cows that long chain FA intermediates formed during BH of specific FA in fish oil are incorporated into milk fat triacylglycerols (Kairenius et al., 2015). However, the relative abundance of *trans*-20 and 22-carbon BH intermediates in milk from cows and goats differed that probably reflects differences in the amount and composition of fish oil fed in the current and a previous investigation (Kairenius et al., 2015). Based on previous studies (Offer et al., 1999; Gama et al., 2008; Toral et al., 2014), it was anticipated that the FO treatment would result in distinct differences in milk fat synthesis in cows and goats. However, FO decreased milk fat content in both species in the absence of alterations in milk yield or milk protein content (Table 2). Whereas the FO treatment did not lower daily milk fat yield (g/d) in goats, secretion of milk fat was lower when expressed on a kilogram of BW basis (−21%), but to a lesser extent than for cows (−29%; Figure 1). Several studies have demonstrated that dietary fish oil supplements do

not induce MFD in goats (Gagliostro et al., 2006; Toral et al., 2014). Part of the reason for differences in milk fat responses to FO between the present study and earlier investigations may be related to a higher combined intake of 20:5n-3 and 22:6n-3 (0.58 vs. 0.12–0.28% diet DM, respectively). When fed relatively high amounts of very long-chain PUFA, goats may experience MFD. In dairy ewes, a lower combined intake of 20:5n-3 and 22:6n-3 (~0.20–0.24% diet DM; Toral et al., 2010; Bichi et al., 2013) induced MFD (up to −21% decrease in milk fat content), providing further evidence of interspecies differences in the lipid metabolism of ruminants.

Examination of changes in milk FA profile, in particular the concentration of 18-, 20-, and 22-carbon BH intermediates, suggest that the FA in FO increased greater disturbances of rumen lipid metabolism in cows compared with goats, which may potentially explain the differences in the severity of MFD between species. Concentrations of *trans*-10 18:1, *trans* 18:2, *trans* 18:3, and *trans* 20- and 22-carbon unsaturated FA were higher in milk of cows than goats on the FO treatment (with the exception of *trans*-11 18:1, *trans*-9,*cis*-11 CLA, *trans*-6,*trans*-12,*trans*-17 20:3, and *trans*-9,*trans*-14,*trans*-17 20:3 that were increased to a similar extent in both species, and *cis*-9,*trans*-11 CLA and *trans*-6,*cis*-11,*cis*-14,*cis*-17 20:4 that were more abundant in goat milk), whereas the abundance of *trans*-10,*cis*-12 CLA was not altered in either species. Consistent with previous studies, an increase in milk *trans*-10,*cis*-12 CLA concentration (Loor et al., 2005a; Shingfield and Griinari, 2007; Toral et al., 2010) or greater accumulation of *trans*-10,*cis*-12 CLA in the rumen (Shingfield and Griinari, 2007; Boeckaert et al., 2008) is not a common feature of MFD in ruminants fed marine lipids.

Fish oil-induced MFD in goats was associated with the highest enrichment of *trans*-9,*cis*-11 CLA (0.15% of total milk FA; Figure 2), a BH intermediate reported to inhibit milk fat synthesis in cows (Harvatine et al., 2009). Concentrations of *trans*-9,*cis*-11 CLA are known to be increased in milk of cows (Loor et al., 2005a; Boeckaert et al., 2008) and ewes (Toral et al., 2010) fed marine lipids or in cows fed high-starch diets containing 18:2n-6 (Roy et al., 2006), but enrichment in these studies was lower than the concentration in milk (0.38% of total milk FA) used to demonstrate an effect on milk fat secretion in cows (Harvatine et al., 2009; Shingfield et al., 2010). Whereas no association existed between decreases in milk fat content and increases in milk *trans*-9,*cis*-11 CLA concentrations to FO in goats ($r = -0.259$, $P = 0.183$, $n = 14$), a significant relation was observed in cows ($r = -0.749$, $P < 0.001$, $n = 12$). However, little compelling evidence exists that increased ruminal formation of *trans*-9,*cis*-11 CLA is a major contributor to MFD on diets containing marine lipid supplements in ruminants. Other intermediates or mechanisms may be involved which require further investigation.

Compared with milk from goats, bovine milk contained higher concentrations of 10-O-16:0, 10-O-18:0, and 13-O-18:0, products formed during sequential hydration and oxidation of dietary FA in the rumen (Jenkins et al., 2008). Marine lipids are known to enrich the concentration of oxygenated FA in milk of ruminants (Márquez-Ruiz et al., 2011; Bichi et al., 2013; Toral et al., 2014), but the possible physiological effects of hydroxy and keto acids in ruminants are not known (Raphael et al., 2014).

For both cows and goats, the decrease in milk fat content on the FO treatment was associated with an increase in milk fat *cis*-11 18:1 concentration ($r = -0.837$, $n = 12$, $P < 0.001$, and $r = -0.639$, $n = 14$, $P < 0.001$, respectively). Earlier reports have also identified a negative relation between these parameters in cows fed supplements of fish oil alone (Gama et al., 2008; Kairenius et al., 2015) or as a mixture with sunflower oil (Shingfield et al., 2006), and in goats fed supplements of extruded linseed and fish oil (Bernard et al., 2015). Incubations with *cis*-11 18:1 were shown to decrease lipogenesis in bovine adipocytes (Burns et al., 2012), whereas postruminal infusion of a mixture of 18:1 isomers containing *cis*-11 18:1 had no effect on milk fat synthesis in cows (Shingfield et al., 2007).

Common changes in milk FA during MFD to FO in both species may offer an insight into the mechanisms responsible for the inhibition of milk fat secretion. In addition to the substantial increase in several *trans* FA isomers, the fish oil-induced MFD phenotype was characterized by a marked decrease in milk 18:0 and *cis*-9 18:1 concentrations. Indirect comparisons suggest that supplements of fish oil (<20 g/kg of diet DM) had a smaller effect on milk 18:0 and *cis*-9 18:1 concentrations in goats (Toral et al., 2014) than in cows and ewes (e.g., Offer et al., 1999; Loor et al., 2005a; Toral et al., 2010). In the present study, the FO treatment resulted in much larger decreases in milk 18:0 and *cis*-9 18:1 concentrations in goats (Figure 2) than expected, possibly due to the higher intakes of 20:5n-3, 22:5n-3, and 22:6n-3 compared with earlier reports. A decrease in 18:0 and *cis*-9 18:1 concentrations is a consistent feature of changes in milk fat composition to marine lipids in cows (Loor et al., 2005a; Boeckaert et al., 2008; Gama et al., 2008), ewes (Toral et al., 2010; Bichi et al., 2013), and goats (Gagliostro et al., 2006; Toral et al., 2014). Based on the well-characterized changes in milk FA composition and associated MFD in cows and sheep fed diets containing marine lipids, it has been suggested that factors other than direct inhibition by specific *trans* FA, including regulation of triacylglycerols synthesis to

maintain milk fat fluidity, may explain, or at least contribute to, the decrease in milk fat synthesis (Loor et al., 2005a; Shingfield and Griinari, 2007; Gama et al., 2008; Toral et al., 2010). Conversion of 18:0 (melting point: 69°C) to *cis*-9 18:1 (melting point: 14°C) through the action of Δ^9-desaturase and selective esterification of 4- to 10- carbon FA and *cis*-9 18:1 to glycerol at sn-3 are important mechanisms ensuring adequate milk fat fluidity (Timmen and Patton, 1988; Chilliard et al., 2000). Milk fat content is positively associated with milk fat 18:0 percentage in goats (Bernard et al., 2006). A shortage of 18:0 for *cis*-9 18:1 synthesis in the mammary gland, together with higher availability of *trans* FA (with melting points above body temperature) that may lead to an overall increase in milk fat melting point, has been proposed as a mechanism to explain fish oil-induced MFD in lactating cows (Loor et al., 2005a; Shingfield and Griinari, 2007; Gama et al., 2008; Harvatine et al., 2009). Previous investigations in cows have reported that fish oil may increase (Gama et al., 2008) or have no influence (Kairenius et al., 2015) on calculated mean melting point of FA in cows. No effects of fish oil on mean milk fat melting point have been reported in goats (Toral et al., 2014). In the present study, the FO treatment lowered calculated milk FA melting point in both species, but these estimates assume the melting points of individual FA in milk are additive and ignores the nonrandom esterification of FA to glycerol (Toral et al., 2013). Furthermore, the necessity to maintain milk fat melting point may introduce a higher requirement for FA synthesized de novo to facilitate the translocation and export of triacylglycerols from the mammary secretory cell. If such requirements cannot be met, the corollary is a decrease in triacylglycerol synthesis to accommodate changes in preformed FA supply to ensure efficient ejection of fat from the mammary gland. Milk fat *cis*-9 18:1/18:0 concentration ratio was increased by 50% on the FO treatment in both species (Table 3). Studies in cows (Loor et al., 2005a; Gama et al., 2008: Kairenius et al., 2015), ewes (Bichi et al., 2013), and goats (Gagliostro et al., 2006; Toral et al., 2014) have also demonstrated an increase in this ratio in response to marine lipid supplements. An increase in this ratio is most probably explained by lowered 18:0 availability rather than elevated Δ^9-desaturase catalyzed conversion of 18:0 to *cis*-9 18:1, given that other desaturation ratios in milk were unaffected (Table 3), *cis*-9 14:1/14:0 in particular, which is considered a suitable proxy of mammary Δ^9-desaturase activity (Bernard et al., 2013). Collectively, these considerations do not implicate changes in Δ^9-desaturase enzyme activity as being a major contributor to MFD in goats and cows fed the FO treatment.

Milk LPL Activity

No obvious association was noted between changes in milk fat synthesis to dietary treatments with milk LPL activity in either species. Irrespective of diet, LPL activity was consistently higher in milk of cows compared with goats (Table 2), in agreement with earlier reports in the literature (Chilliard et al., 2003). However, milk LPL activity was more responsive to lipid supplements in goats than cows (Table 2). In goats, the SOS treatment lowered milk LPL activity, which is typical when dietary supplements of plant oils and oilseeds are fed that may also alter milk sensory quality by lowering the development of goat flavor (Chilliard et al., 2003; Bernard et al., 2005; Eknæs et al., 2009). In goats, FO caused greater decreases in milk LPL activity, whereas feeding lower amounts of fish oil had no effect on free FA concentrations postmilking (Toral et al., 2014), suggesting a dose-dependent relation between amount of fish oil in the diet and milk LPL activity.

CONCLUSIONS

Direct comparison of milk fat yield to diets formulated to induce MFD in cows and goats provides further support for the BH theory to explain species differences in milk fat content and yield on high-starch diets containing plant oils. However, increases in milk *trans*-10,*cis*-12 CLA concentration do not, in isolation, appear to fully explain MFD on the SOS treatment in cows, suggesting that other BH intermediates with potential antilipogenic effects including *trans*-9,*cis*-11 CLA and *trans*-10 18:1 may also be involved. Comparison of milk fat composition from cows and goats fed the SOS treatment demonstrated major interspecies differences in mammary lipogenesis, including differences in Δ^9-desaturation ratios and a lower sensitivity to the inhibitory effects of *trans*-10,*cis*-12 CLA in goats than cows and by inference in ruminal lipid metabolism (i.e., being more stable and robust to alterations in diet composition with a less pronounced *trans*-10 shift in the goat compared with the cow). However, some changes in milk FA to the SOS treatment were similar between species, including comparable increases in *trans*-10,*cis*-12 CLA, *trans*-10,*trans*-12 CLA, and 10-O-18:0 concentration in milk. Dietary supplements of a relatively high dose of fish oil (22 g/kg of diet DM) resulted in fewer species differences than anticipated, demonstrating that marine lipids may also induce MFD, to some extent, in the goat. Changes in milk fat composition to FO in both species were characterized by decreases in 18:0 and *cis*-9 18:1 and an increase in 16- to 22-carbon *trans* FA. Even though FO lowered

the calculated mean milk FA melting point, the necessity to accommodate changes in preformed FA supply to maintain milk fat fluidity may contribute to a decrease in triacylglycerol synthesis in ruminants fed diets containing fish oil. Fish oil elevated *trans*-9,*cis*-11 CLA concentrations and the abundance of other BH intermediates, including *trans*-10 18:1, *cis*-11 18:1, and 20- and 22- carbon FA containing a *trans*-10 double bond that may also contribute to fish oil-induced MFD.

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