

## Effect of plant oils in the diet on performance and milk fatty acid composition in goats fed diets based on grass hay or maize silage

Laurence Bernard<sup>1</sup>, Kevin J. Shingfield<sup>2</sup>, Jacques Rouel<sup>1</sup>, Anne Ferlay<sup>1</sup> and Yves Chilliard<sup>1\*</sup>

<sup>1</sup>INRA, UR1213 Herbivores, Equipe Tissu Adipeux et Lipides du Lait, Site de Theix, F-63122 Saint-Genès-Champanelle, France

<sup>2</sup>Animal Production Research, MTT Agrifood Research Finland, Jokionen FIN 31600, Finland

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Based on the potential benefits to long-term human health there is interest in developing sustainable nutritional strategies for reducing saturated and increasing specific unsaturated fatty acids in ruminant milk. The impact of plant oil supplements to diets containing different forages on caprine milk fatty acid composition was examined in two experiments using twenty-seven Alpine goats in replicated 3 × 3 Latin squares with 28 d experimental periods. Treatments comprised of no oil (control) or 130 g/d of sunflower-seed oil (SO) or linseed oil (LO) supplements added to diets based on grass hay (H; experiment 1) or maize silage (M; experiment 2). Milk fat content was enhanced ( $P < 0.01$ ) on HSO, HLO and MLO compared with the corresponding H or M control diets, resulting in 17, 15 and 14% increases in milk fat secretion, respectively. For both experiments, plant oils decreased ( $P < 0.05$ ) milk 10:0–16:0 and odd- and branched-chain fatty acid content and increased 18:0, *trans*- $\Delta^{6-9,11-14,16-18}$ :1 (and their corresponding  $\Delta$ -9 desaturase products), *trans*-7,*trans*-9-conjugated linoleic acid (CLA), *trans*-9,*trans*-11-CLA and *trans*-8,*cis*-10-CLA concentrations. Alterations in the distribution of *cis*-18:1, *trans*-18:1, -18:2 and CLA isomers in milk fat were related to plant oil composition and forage in the diet. In conclusion, plant oils represent an effective strategy for altering the fatty acid composition of caprine milk, with evidence that the basal diet is an important determinant of ruminal unsaturated fatty acid metabolism in the goat.

### Plant oils: Goats' milk: Conjugated linoleic acid: *Trans*-fatty acids

Despite the lower scale of milk production from goats compared with cows in Europe, there is increasing interest in caprine milk due to inherent species-specific biochemical properties that contribute to nutritional quality. Caprine milk has been identified as a viable alternative for consumers that are sensitive or develop allergic reactions to bovine milk, and is known to be beneficial with respect to Cu, Zn and Se bioavailability<sup>(1)</sup>. Lipid in goats' milk is more digestible than bovine milk fat which may be related to the lower mean milk fat globule size, higher 8:0–10:0 concentrations and a larger proportion of short- and medium-chain fatty acids esterified at *sn*-3 in milk fat TAG<sup>(2)</sup>.

Fatty acid composition is an important determinant of milk nutritional quality, with evidence that certain specific fatty acids exert negative effects (12:0, 14:0, 16:0) when consumed in excess, whilst others have potentially beneficial effects (*anteiso*-15:0, *cis*-9-18:1, 18:3*n*-3) on human health<sup>(3)</sup>. Furthermore, there is evidence in animal models that the predominant isomer of conjugated linoleic acid (CLA) in ruminant milk, *cis*-9,*trans*-11, exhibits anticarcinogenic and anti-atherogenic

properties<sup>(4)</sup>. Nutritional strategies for enhancing CLA content in caprine milk also result in an inevitable increase in *trans*-18:1 concentrations, with *trans*-11 typically being the major isomer<sup>(5)</sup>. Epidemiological studies have implicated high intakes of *trans*-18:1 and -18:2 in the human being associated with an increase in CVD risk<sup>(6)</sup>, with emerging data supporting isomer-specific effects<sup>(7)</sup>. A detailed evaluation of milk fatty acid composition responses to nutritional factors in the goat is therefore essential in attempting to establish the possible role of foods derived from modified caprine milk on long-term human health and disease prevention.

Nutrition is the main environmental factor regulating milk fat synthesis and fatty acid composition in ruminants<sup>(8)</sup>. However, experimental evidence on the role of diet on milk fat composition in the goat is limited<sup>(5,9)</sup> whereas the nutritional regulation of bovine milk fat composition has been extensively investigated<sup>(5,7,10,11)</sup>. For both species, forage in the diet is known to affect milk fat composition responses to plant oils, including *trans*-18:1 and CLA isomer concentrations<sup>(5,10)</sup>. Nutritional modification of caprine milk fat composition is

**Abbreviations:** CLA, conjugated linoleic acid; FAME, fatty acid methyl esters; H, diet based on natural grassland hay (experiment 1) supplemented with no additional oil; HLO, diet based on natural grassland hay (experiment 1) supplemented with linseed oil; HSO, diet based on natural grassland hay (experiment 1) supplemented with sunflower-seed oil; M, diet based on maize silage (experiment 2) supplemented with no additional oil; MLO, diet based on maize silage (experiment 2) supplemented with linseed oil; MSO, diet based on maize silage (experiment 2) supplemented with sunflower-seed oil; PC, principal component; PCA, principal component analysis.

\* **Corresponding author:** Dr Yves Chilliard, fax +33 473624519, email Yves.Chilliard@clermont.inra.fr

often accompanied by changes in milk fat synthesis, responses that differ compared with the lactating cow. Inclusion of lipids in the diet enhances milk fat secretion in the goat in the absence of systematic changes in milk yield and protein content<sup>(5,9)</sup>. In contrast, lipid supplements in the lactating cow typically increase milk production and tend to reduce milk protein content, whereas decreases in fat content occur on high-concentrate diets containing plant oils or in response to marine oils<sup>(12,13)</sup>. The underlying mechanisms accounting for these differences are not well established but may reflect inter-species differences in digestion and/or specific metabolic responses.

*Cis-9,trans-11-CLA* in milk is derived from the ruminal metabolism of 18:2*n-6*<sup>(14)</sup> and endogenous synthesis via the action of  $\Delta$ -9 desaturase on *trans-11-18:1* in ruminant tissues<sup>(15,16)</sup>. *Trans-11-18:1* is a common intermediate of 18:2*n-6* and 18:3*n-3* metabolism in the rumen<sup>(17)</sup>. Studies in the lactating goat have been confined, in the most part, to evaluating the changes in major milk fatty acid concentrations to lipids rich in 18:3*n-3*<sup>(5,9)</sup>. Experiments with lactating cows have established that the forage source in the diet is a major determinant of milk fat composition responses to oils rich in unsaturated fatty acids<sup>(18,19)</sup>, whereas few data are available in goats. The present study was designed to examine the impact of plant oils on milk production and milk fatty acid composition in goats fed diets containing forages of differing botanical and chemical composition. A comprehensive evaluation of the effects of experimental treatments on milk fat composition, with specific emphasis on *trans-18:1*, 18:2 and CLA isomers, also provided further insight into the possible role of biohydrogenation intermediates in the regulation of milk fat synthesis in the goat.

## Materials and methods

### *Animals, management and experimental design*

All experimental procedures were approved by the Animal Care Committee of INRA in accordance with the Use of Vertebrates for Scientific Purposes Act 1985. Animals were recruited to experiments and allocated to treatment groups according to milk yield, milk fat and protein content, parity, stage of lactation and genotype score at the  $\alpha S1$  casein locus. Goats of the 'middle type'  $\alpha S1$  casein genotype<sup>(20)</sup> content were used in both experiments since this is associated with effects on milk traits<sup>(21)</sup> and fatty acid composition<sup>(22)</sup>. For the first experiment, thirteen multiparous (3.2 (SD 1.3)) Alpine goats in mid-lactation (77 (SD 7)) d in lactation) were offered three experimental diets according to a replicated 3 × 3 Latin square design with 28 d experimental periods using four or five animals per group. In the second experiment, fourteen multiparous (3.3 (SD 1.1)) Alpine goats in mid-lactation (70 (SD 7)) d in lactation) were fed three experimental diets according to a replicated 3 × 3 Latin square design with four or five animals per group with 28 d experimental periods. Fifteen goats were recruited for each experiment, but due to mammary cysts or pseudo-pregnancy, two animals were withdrawn from the first experiment and one from the second. Each experimental period was comprised of 21 d adaptation and 7 d sampling periods. Goats were housed in a metabolism unit in individual stalls, with continuous access to water and milked at 08.00 and

16.00 hours. For both experiments, diets were formulated to meet energy and protein requirements<sup>(23)</sup>.

### *Experimental diets*

In experiment 1, diets were comprised of hay prepared from regrowths of natural grassland pasture offered *ad libitum* and a concentrate mixture (Table 1) supplemented with no additional lipid (H), linseed oil (130 g/d) (HLO; SA Vandeputte, Mouscron, Belgium) or sunflower-seed oil (130 g/d) (HSO; Auvergne Trituration, Lezoux, France). For experiment 2, diets were based on maize silage offered *ad libitum* and a concentrate mixture (Table 1) supplemented with no additional lipid (M), sunflower-seed oil (130 g/d) (MSO) or linseed oil (130 g/d) (MLO). In both experiments, concentrates allocated according to milk yield at the start of experiment and plant oils were mixed together and offered as two equal meals at 08.30 and 16.30 hours. Experiments were conducted during the Spring (from March until the end of May).

### *Measurements and sampling*

Individual intakes were recorded daily, but only measurements collected during the last week of each experimental period were used for statistical analysis. During each experimental period, representative samples of hay, maize silage and concentrates were composited daily and stored at -20°C. Chemical composition of feed ingredients was determined using standard procedures<sup>(24)</sup>. Milk yields of individual goats were recorded thrice weekly, while only measurements collected during the last week of each experimental period were analysed statistically. Samples of milk for the measurement of fat, protein and lactose were collected from each goat over four consecutive milkings starting at 08.00 hours on day 21 of each experimental period and treated with preservative (potassium bichromate; Merck, Fontenay-Sous-Bois, France). Milk fat, protein and lactose were determined by near IR spectroscopy<sup>(24)</sup> calibrated using reference caprine milk samples. Unpreserved samples of milk were collected over two consecutive milkings starting at 08.00 hours on day 22 of each experimental period, stored at -20°C, composited and submitted for fatty acid analysis. Live weight of experimental animals was measured at the start and end of each experimental period in both experiments.

### *Lipid analysis*

Lipids in natural grassland hay, maize silage and concentrates were extracted<sup>(25)</sup> and transesterified<sup>(26)</sup> according to standard procedures using 23:0 (Sigma, Saint-Quentin Fallavier, France) as an internal standard. For experiment 1, lipids in 130 mg of lyophilised milk samples were extracted in 10 ml of a mixture of hexane–diethyl ether (50:50, v/v), 1 ml saturated NaCl solution and 1 ml ethanol. After mixing, the organic phase was recovered by centrifugation at 1000 rpm for 10 min at 4°C, repeatedly rinsed (*n* 3) with 5 ml of a mixture of hexane–diethyl ether (50:50, v/v) and dried under N<sub>2</sub>. Fatty acid methyl esters (FAME) were prepared following the addition of 100 µl of 1 M-sodium methanolate at room temperature for 10 min followed by 500 µl of 14% (v/v) boron trifluoride in methanol for 10 min<sup>(27)</sup>. Lipids in 130 mg

**Table 1.** Ingredient and chemical composition of experimental diets

Treatment...	Experiment 1			Experiment 2		
	H	HSO	HLO	M	MSO	MLO
Ingredient (g/kg DM)						
Natural grassland hay	444	484	486	0.0	0.0	0.0
Maize silage	0.0	0.0	0.0	388	455	452
Sunflower-seed oil*	0.0	55	0.0	0.0	61	0.0
Linseed oil†	0.0	0.0	55	0.0	0.0	62
Rolled barley	197	112	111	249	118	118
Dehydrated sugarbeet pulp	227	179	178	244	163	163
Pelleted dehydrated lucerne	65	54	54	0.0	0.0	0.0
Soyabean meal	67	116	116	119	203	205
Minerals and vitamins‡	0.04	0.04	0.04	0.05	0.05	0.05
Chemical composition (g/kg DM)						
Organic matter	913	857	856	945	885	883
Crude protein	167	183	183	140	171	171
Neutral-detergent fibre	421	407	406	310	295	294
Acid-detergent fibre	213	209	209	174	171	171
Starch	124	69	69	280	221	220
Ether extract	23	80	81	20	82	84
14:0	0.15	0.16	0.16	0.05	0.09	0.08
16:0	4.3	7.9	7.6	4.1	8.1	7.7
<i>cis</i> -9-16:1	0.1	0.1	0.1	0.1	0.1	0.1
18:0	0.4	2.2	1.3	0.4	2.8	2.7
<i>cis</i> -9-18:1	1.4	12.7	10.7	3.5	14.5	15.6
<i>cis</i> -11-18:1	0.2	0.8	0.8	0.2	0.6	0.7
18:2 <i>n</i> -6	5.1	41.4	13.2	9.3	52.7	19.4
18:3 <i>n</i> -3	6.0	6.4	37.7	1.5	1.7	31.9
Σ Fatty acids	19	75	75	20	82	83

H, diet based on natural grassland hay (experiment 1) supplemented with no additional oil; HSO, diet based on natural grassland hay (experiment 1) supplemented with sunflower-seed oil; HLO, diet based on natural grassland hay (experiment 1) supplemented with linseed oil; M, diet based on maize silage (experiment 2) supplemented with no additional oil; MSO, diet based on maize silage (experiment 2) supplemented with sunflower-seed oil; MLO, diet based on maize silage (experiment 2) supplemented with linseed oil.

\*Sunflower-seed oil in experiment 1 contained (g/kg): 16:0, 64.7; 18:0, 30.8; *cis*-9-18:1, 204.5; 18:2*n*-6, 657.2; 18:3*n*-3, 0.0. Sunflower-seed oil in experiment 2 contained (g/kg): 16:0, 66.3; 18:0, 38.3; *cis*-9-18:1, 174.9; 18:2*n*-6, 704.3; 18:3*n*-3, 0.9.

†Linseed oil in experiment 1 contained (g/kg): 16:0, 58.2; 18:0, 16.0; *cis*-9-18:1, 168.3; 18:2*n*-6, 147.1; 18:3*n*-3, 566.8. Linseed oil in experiment 2 contained (g/kg): 16:0, 58.4; 18:0, 35.5; *cis*-9-18:1, 189.4; 18:2*n*-6, 155.8; 18:3*n*-3, 489.7.

‡Mineral-vitamin supplement declared as containing (g/kg): Ca, 240; P, 60; Mg, 50; Na, 15; Zn, 7; Mn, 6; α-tocopherol, 0.3; retinol, 0.2; cholecalciferol, 0.002 (Centraliment, Ussel France).

of lyophilised milk samples from experiment 2 were methylated directly using 2 ml 0.5 M-sodium methanolate at 50°C for 5 min, followed after cooling by the addition of 100 µl 12 M-HCl at room temperature for 10 min. FAME were recovered in 2 ml hexane and washed with 3 ml water. Direct comparisons indicated no differences in FAME profile between transesterification procedures applied to samples from experiments 1 and 2<sup>(22)</sup>.

Methyl esters were quantified by GLC using a gas chromatograph Trace-GC 2000 equipped with a flame-ionisation detector (Thermo Finnigan, Les Ullis, France) and 100 m fused silica capillary column (CP-SIL 88; Chrompack 7489, Middelburg, The Netherlands) using H<sub>2</sub> as the carrier and fuel gas. Total FAME profile in a 2 µl sample at a split ratio of 1:50 was determined using a temperature gradient program<sup>(26)</sup>. Peaks were routinely identified using authentic standards (Sigma, Saint-Quentin Fallavier, France) and a reference butter oil (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain (4:0–10:0) fatty acids. Methyl esters not contained in commercially available standards were identified based on comparisons with reference milk fat samples for which detailed structural analysis was made based on GC-MS of 4,4-dimethyloxazoline fatty acid derivatives<sup>(7,28)</sup>.

The distribution of CLA isomers in milk FAME was determined by HPLC using four silver-impregnated silica columns (ChromSpher 5 lipids, 250 × 4.6 mm, 5 µm particle size; Varian Ltd, Walton-on-Thames, Surrey, UK) coupled in series and 0.1 % (v/v) acetonitrile in heptane as the mobile phase<sup>(29)</sup>. Isomers were identified using an authentic CLA methyl ester standard (O-5632; Sigma-Aldrich, YA-Kemia Limited, Helsinki, Finland) and chemically synthesised *trans*-9,*cis*-11-CLA<sup>(18)</sup>. Identification was verified by cross-referencing with the elution order reported in the literature<sup>(29)</sup> using *cis*-9,*trans*-11-CLA as a landmark isomer.

#### Statistical analysis

Experimental data from both experiments were subjected to ANOVA using the general linear model procedure of the SAS software package (version 8.2; SAS Institute, Cary, NC, USA) with a model that included the effects of goat, period, and treatment. Least-square means with their standard errors are reported and treatment effects were declared significant at  $P < 0.05$ . Relationships between oil treatments, forages and milk production and composition were assessed by principal component analysis (PCA), using dedicated on-line software ('R' software package; <http://www.r-project.org/>).

## Results

### Diet composition

Maize silage was of high quality both in terms of nutritive value and fermentation characteristics and had the following composition (g/kg DM, unless otherwise stated): DM (g/kg fresh weight), 308; organic matter, 949; crude protein, 86; acid-detergent fibre, 243; neutral-detergent fibre, 412; starch, 327; fatty acid content, 29. Natural grassland hay was of high nutritional quality and had the following composition (g/kg DM, unless otherwise stated): DM (g/kg fresh weight), 827; organic matter, 899; crude protein, 187; acid-detergent fibre, 277; neutral-detergent fibre, 548; fatty acid content, 26.

Crude protein, starch and neutral-detergent fibre content of H-based diets averaged 178, 87 and 411 g/kg DM with corresponding values for M-based diets of 161, 240 and 300 (Table 1). Grassland hay and linseed oil were rich in 18:3n-3 (11.9 and 567 g/kg DM), while 18:2n-6 predominated in maize silage, concentrates and sunflower-seed oil (12.3, 8.2 and 704 g/kg DM, respectively).

### Animal performance

Plant oils reduced ( $P<0.05$ ) DM intake on M-based diets (experiment 2). Irrespective of the basal forage, linseed oil and sunflower-seed oil in the diet, with the exception of treatment MSO, had no effect on milk yield. Inclusion of plant oils increased ( $P<0.01$ ) milk fat content and yield in diets based on natural grassland hay, while milk fat content was enhanced for MLO compared with M and MSO treatments (Table 2). In both experiments, plant oils enhanced ( $P<0.001$ ) lactose content (Table 2), but only treatments MSO and MLO increased ( $P<0.001$ ) milk lactose secretion compared with the M control diet.

Energy and N balances<sup>(23)</sup> were positive in both experiments (data not shown), with mean values of 1044 and 2160 kJ net energy for lactation per d, and 57 and 30 g digestible protein at the intestine per d, for diets fed in experiments 1 and 2, respectively.

### Milk fatty acid composition

In both experiments, plant oils in the diet increased ( $P<0.05$ ) milk 4:0 and decreased ( $P<0.05$ ) 8:0 concentrations (Table 3). Changes in milk fatty acid composition to sunflower-seed oil and linseed oil were characterised by decreases ( $P<0.05$ ) in milk fat 9:0 to 17:0 and branched-chain fatty acids, except *iso*-17:0, and an increase in 18:0 concentration, responses that were comparable between experiments (Table 3). Concentrations of 18:3n-3 were reduced ( $P<0.05$ ) in response to sunflower-seed oil on hay-based diets (experiment 1), but were enhanced ( $P<0.05$ ) by linseed oil in maize silage-based diets (experiment 2). Milk fat 20:5n-3 and 22:5n-3 concentrations were higher in experiment 1 compared with experiment 2, whilst sunflower-seed oil and linseed oil in the diet tended to decrease milk 20:5n-3 content (Table 3). In both experiments, plant oils also decreased ( $P<0.05$ ) *cis*-9-10:1, *cis*-9-14:1 and *cis*-9-16:1 concentrations and resulted in an overall relative reduction in milk 4:0–16:0 concentration of 34, 37, 36 and 34%, for treatments HSO, MSO, HLO and MLO, respectively.

Concentrations of *trans*-18:1 isomers were increased ( $P<0.001$ ) by plant oils in both experiments (Table 3) with higher concentrations in milk from diets based on maize silage (experiment 2) compared with natural grassland hay (experiment 1). Relative increases in milk fat *trans*-18:1 to sunflower-seed oil were higher for MSO (+500%) than HSO (+390%) treatments. Irrespective of forage type, plant oils enhanced ( $P<0.05$ ) milk *trans*- $\Delta^{4-9}$  concentrations, while increases ( $P<0.01$ ) in *trans*-10-18:1 content to sunflower-seed oil were confined to maize silage-based diets (+630%). Plant oils enhanced ( $P<0.05$ ) milk *trans*-11-18:1 concentrations in both experiments, but the relative increases to linseed oil in the diet were larger for HLO (+439%) than MLO (+358%) treatments (Table 4). Overall, *trans*-11 accounted for 48–59 and 67–82% of total *trans*-18:1 in milk for M- or H-based diets. Inclusion of sunflower-seed oil, and to a larger extent linseed oil, increased ( $P<0.05$ ) *trans*-13,14-18:1 concentrations, responses that

**Table 2.** Effect of experimental treatment on DM intake, milk yield and milk composition (Mean values with their standard errors for thirteen goats (experiment 1) and fourteen goats (experiment 2))

	Experiment 1				Experiment 2			
	H	HSO	HLO	SEM*	M	MSO	MLO	SEM*
DM intake (kg/d)	2.28	2.25	2.23	0.043	2.21 <sup>b</sup>	2.06 <sup>a</sup>	2.03 <sup>a</sup>	0.027
Yield (g/d)								
Milk	3340	3320	3300	41.6	3370 <sup>a</sup>	3620 <sup>b</sup>	3470 <sup>a,b</sup>	0.057
Fat	109 <sup>a</sup>	128 <sup>b</sup>	125 <sup>b</sup>	1.869	107 <sup>a</sup>	114 <sup>a</sup>	122 <sup>b</sup>	2.620
Protein	99	99	99	1.379	102 <sup>a</sup>	109 <sup>b</sup>	106 <sup>a,b</sup>	1.932
Lactose	153	157	156	1.828	157 <sup>a</sup>	176 <sup>b</sup>	170 <sup>b</sup>	2.842
Concentration (g/kg)								
Fat	32.3 <sup>a</sup>	37.9 <sup>b</sup>	37.4 <sup>b</sup>	0.517	31.4 <sup>a</sup>	31.6 <sup>a</sup>	35.3 <sup>b</sup>	0.743
Protein	29.6 <sup>a</sup>	30.1 <sup>a,b</sup>	30.4 <sup>b</sup>	0.148	30.4	30.2	30.8	0.280
Lactose	45.5 <sup>a</sup>	47.2 <sup>b</sup>	46.9 <sup>b</sup>	0.228	46.5 <sup>a</sup>	48.5 <sup>b</sup>	48.8 <sup>b</sup>	0.289

H, diet based on natural grassland hay (experiment 1) supplemented with no additional oil; HSO, diet based on natural grassland hay (experiment 1) supplemented with sunflower-seed oil; HLO, diet based on natural grassland hay (experiment 1) supplemented with linseed oil; M, diet based on maize silage (experiment 2) supplemented with no additional oil; MSO, diet based on maize silage (experiment 2) supplemented with sunflower-seed oil; MLO, diet based on maize silage (experiment 2) supplemented with linseed oil.

<sup>a,b,c</sup> Mean values for each experiment within a row with unlike superscript letters were significantly different ( $P<0.05$ ).

\* Error df 22 and 24 for experiments 1 and 2, respectively.



**Table 3.** Effect of experimental treatment on milk fatty acid composition (g/100 g fatty acids) (Mean values with their standard errors for thirteen goats (experiment 1) and fourteen goats (experiment 2))

	Experiment 1				Experiment 2			
	H	HSO	HLO	SEM*	M	MSO	MLO	SEM*
4:0	2.27 <sup>a</sup>	2.58 <sup>b</sup>	2.64 <sup>b</sup>	0.056	2.38 <sup>a</sup>	2.56 <sup>a,b</sup>	2.72 <sup>b</sup>	0.074
6:0	2.25 <sup>a</sup>	2.08 <sup>b</sup>	2.17 <sup>a,b</sup>	0.048	2.47	2.22	2.44	0.009
8:0	2.52 <sup>a</sup>	2.01 <sup>c</sup>	2.23 <sup>b</sup>	0.060	2.74 <sup>a</sup>	2.19 <sup>b</sup>	2.54 <sup>a</sup>	0.107
9:0	0.06 <sup>a</sup>	0.04 <sup>b</sup>	0.05 <sup>c</sup>	0.002	0.11 <sup>a</sup>	0.08 <sup>b</sup>	0.09 <sup>a,b</sup>	0.010
10:0	9.48 <sup>a</sup>	6.13 <sup>b</sup>	6.81 <sup>b</sup>	0.243	10.58 <sup>a</sup>	6.91 <sup>b</sup>	8.06 <sup>c</sup>	0.299
<i>cis</i> -9-10:1	0.25 <sup>a</sup>	0.14 <sup>b</sup>	0.15 <sup>b</sup>	0.005	0.27 <sup>a</sup>	0.13 <sup>c</sup>	0.17 <sup>b</sup>	0.011
11:0	0.10 <sup>a</sup>	0.04 <sup>c</sup>	0.06 <sup>b</sup>	0.004	0.20 <sup>a</sup>	0.10 <sup>b</sup>	0.12 <sup>b</sup>	0.017
12:0	5.00 <sup>a</sup>	2.65 <sup>b</sup>	2.94 <sup>b</sup>	0.142	5.72 <sup>a</sup>	3.12 <sup>c</sup>	3.54 <sup>b</sup>	0.106
13:0	0.23 <sup>a</sup>	0.10 <sup>c</sup>	0.12 <sup>b</sup>	0.008	0.31 <sup>a</sup>	0.14 <sup>b</sup>	0.17 <sup>b</sup>	0.015
<i>iso</i> -14:0	0.18 <sup>a</sup>	0.10 <sup>b</sup>	0.10 <sup>b</sup>	0.005	0.13 <sup>a</sup>	0.08 <sup>b</sup>	0.07 <sup>b</sup>	0.004
14:0	11.72 <sup>a</sup>	7.42 <sup>b</sup>	7.59 <sup>b</sup>	0.234	12.07 <sup>a</sup>	8.10 <sup>b</sup>	8.36 <sup>b</sup>	0.151
<i>cis</i> -9-14:1	0.21 <sup>a</sup>	0.10 <sup>b</sup>	0.09 <sup>b</sup>	0.006	0.23 <sup>a</sup>	0.10 <sup>b</sup>	0.11 <sup>b</sup>	0.009
<i>iso</i> -15:0	0.31 <sup>a</sup>	0.24 <sup>b</sup>	0.25 <sup>b</sup>	0.008	0.23 <sup>a</sup>	0.16 <sup>b</sup>	0.16 <sup>b</sup>	0.009
<i>anteiso</i> -15:0	0.60 <sup>a</sup>	0.38 <sup>b</sup>	0.44 <sup>c</sup>	0.013	0.49 <sup>a</sup>	0.33 <sup>b</sup>	0.36 <sup>b</sup>	0.017
15:0	1.39 <sup>a</sup>	0.84 <sup>b</sup>	0.91 <sup>b</sup>	0.029	1.20 <sup>a</sup>	0.76 <sup>b</sup>	0.80 <sup>b</sup>	0.036
<i>iso</i> -16:0	0.44 <sup>a</sup>	0.26 <sup>b</sup>	0.28 <sup>b</sup>	0.011	0.41 <sup>a</sup>	0.24 <sup>b</sup>	0.25 <sup>b</sup>	0.018
16:0	26.36 <sup>a</sup>	16.68 <sup>b</sup>	16.14 <sup>b</sup>	0.398	29.85 <sup>a</sup>	18.78 <sup>b</sup>	18.64 <sup>b</sup>	0.299
<i>cis</i> -9-16:1	0.68 <sup>a</sup>	0.41 <sup>b</sup>	0.38 <sup>b</sup>	0.015	0.78 <sup>a</sup>	0.40 <sup>b</sup>	0.43 <sup>b</sup>	0.022
<i>trans</i> -9-16:1	0.17 <sup>a</sup>	1.01 <sup>b</sup>	0.92 <sup>b</sup>	0.050	0.16 <sup>a</sup>	1.07 <sup>c</sup>	0.66 <sup>b</sup>	0.057
<i>iso</i> -17:0	0.56	0.52	0.52	0.014	0.47 <sup>a</sup>	0.60 <sup>b</sup>	0.46 <sup>a</sup>	0.035
17:0	0.69 <sup>a</sup>	0.44 <sup>b</sup>	0.47 <sup>b</sup>	0.013	0.57 <sup>a</sup>	0.38 <sup>b</sup>	0.32 <sup>b</sup>	0.024
18:0	6.88 <sup>a</sup>	12.50 <sup>b</sup>	11.58 <sup>b</sup>	0.392	4.88 <sup>a</sup>	9.01 <sup>c</sup>	8.15 <sup>b</sup>	0.279
∑ <i>trans</i> -18:1	2.25 <sup>a</sup>	11.03 <sup>b</sup>	10.40 <sup>b</sup>	0.373	2.41 <sup>a</sup>	14.46 <sup>c</sup>	10.24 <sup>b</sup>	0.325
∑ <i>cis</i> -18:1	17.52 <sup>a</sup>	22.09 <sup>b</sup>	19.31 <sup>a</sup>	0.733	14.45 <sup>a</sup>	17.44 <sup>b</sup>	17.83 <sup>b</sup>	0.404
∑ 18:2†	2.69 <sup>a</sup>	3.04 <sup>b</sup>	4.29 <sup>c</sup>	0.094	2.73 <sup>a</sup>	3.71 <sup>b</sup>	6.20 <sup>c</sup>	0.246
∑ CLA	0.97 <sup>a</sup>	4.06 <sup>b</sup>	4.18 <sup>b</sup>	0.232	0.93 <sup>a</sup>	4.70 <sup>c</sup>	3.00 <sup>b</sup>	0.273
18:3 <i>n</i> -3	1.04 <sup>a</sup>	0.57 <sup>b</sup>	1.15 <sup>a</sup>	0.044	0.19 <sup>a</sup>	0.15 <sup>a</sup>	0.69 <sup>b</sup>	0.042
20:0	0.15 <sup>a</sup>	0.17 <sup>b</sup>	0.16 <sup>a</sup>	0.005	0.08 <sup>a</sup>	0.12 <sup>b</sup>	0.09 <sup>a</sup>	0.003
20:4 <i>n</i> -6	0.14 <sup>a</sup>	0.07 <sup>b</sup>	0.06 <sup>b</sup>	0.004	0.17 <sup>a</sup>	0.09 <sup>b</sup>	0.10 <sup>b</sup>	0.008
20:5 <i>n</i> -3	0.07 <sup>a</sup>	0.04 <sup>c</sup>	0.05 <sup>b</sup>	0.002	0.03 <sup>a,b</sup>	0.01 <sup>a</sup>	0.03 <sup>b</sup>	0.004
22:0	0.06 <sup>a</sup>	0.09 <sup>b</sup>	0.06 <sup>a</sup>	0.003	0.02 <sup>a</sup>	0.06 <sup>c</sup>	0.04 <sup>b</sup>	0.003
22:5 <i>n</i> -3	0.12 <sup>a</sup>	0.06 <sup>b</sup>	0.06 <sup>b</sup>	0.002	0.07 <sup>a</sup>	0.03 <sup>b</sup>	0.05 <sup>a,b</sup>	0.010
22:6 <i>n</i> -3	0.02	0.05	0.14	0.001	0.01	0.01	0.01	0.003
∑ SFA	71.26 <sup>b</sup>	55.26 <sup>a</sup>	55.48 <sup>a</sup>	0.824	74.90 <sup>b</sup>	55.93 <sup>a</sup>	57.36 <sup>a</sup>	0.552
∑ MUFA	22.39 <sup>a</sup>	36.19 <sup>c</sup>	33.30 <sup>b</sup>	0.685	18.81 <sup>a</sup>	34.24 <sup>c</sup>	30.36 <sup>b</sup>	0.464
∑ PUFA	5.03 <sup>a</sup>	7.82 <sup>b</sup>	9.70 <sup>c</sup>	0.269	4.12 <sup>a</sup>	8.70 <sup>b</sup>	10.07 <sup>c</sup>	0.315

H, diet based on natural grassland hay (experiment 1) supplemented with no additional oil; HSO, diet based on natural grassland hay (experiment 1) supplemented with sunflower-seed oil; HLO, diet based on natural grassland hay (experiment 1) supplemented with linseed oil; M, diet based on maize silage (experiment 2) supplemented with no additional oil; MSO, diet based on maize silage (experiment 2) supplemented with sunflower-seed oil; MLO, diet based on maize silage (experiment 2) supplemented with linseed oil; CLA, conjugated linoleic acid.

<sup>a,b,c</sup> Mean values for each experiment within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* Error df 22 and 24 for experiments 1 and 2, respectively.

† Sum of 18:2 fatty acids excluding isomers of CLA.

were more pronounced for diets based on maize silage compared with grassland hay.

Concentrations of total *cis*-18:1 increased ( $P < 0.001$ ) in response to lipid-supplements (+21 and +23% to sunflower-seed oil and linseed oil, respectively in experiment 2), whereas significant increases were confined to sunflower-seed oil (+26%) in experiment 1 (Table 3). Overall, *cis*-9-18:1 accounted for 85.5–96.2% of total *cis*-18:1. For both experiments, inclusion of plant oils in the diet enhanced *cis*- $\Delta^{12-16}$ -18:1 content (Table 4).

Plant oils altered the relative abundance of non-methylene-interrupted 18:2 isomers (Table 5). In both experiments, sunflower-seed oil and linseed oil increased ( $P < 0.001$ ) milk fat *cis*-9,*trans*-13-18:2 concentrations. Irrespective of the basal diet, linseed oil enhanced ( $P < 0.001$ ) *cis*-9,*trans*-12-18:2, *trans*-9,*cis*-12-18:2, *trans*-9,*trans*-12-18:2, *trans*-11,*cis*-15-18:2 and *trans*-11,*trans*-15-18:2 concentrations in milk fat, while sunflower-seed oil increased ( $P < 0.001$ ) milk

*trans*-9,*trans*-14-18:2 content (Table 5). Concentrations of 18:2*n*-6 were higher ( $P < 0.05$ ) in milk from MSO, and were consistently lower in milk from animals on diets containing linseed oil (Table 5).

#### Milk conjugated 18:2 isomers

In both experiments, plant oils enhanced ( $P < 0.001$ ) *cis*-9,*trans*-11 and total CLA concentrations (Table 5). *Cis*-9,*trans*-11 was the major isomer accounting for 79–91% of total CLA, but a wide range of other CLA isomers were detected in milk, which based on relative abundance ( $> 50$  mg/100 g fatty acids) ranked as *trans*-11,*cis*-13-CLA, *trans*-7,*cis*-9-CLA, *trans*-8,*cis*-10-CLA and *trans*-9,*trans*-11-CLA. Irrespective of the basal forage, plant oils increased ( $P < 0.05$ ) *trans*-9,*trans*-11-CLA and *trans*-7,*trans*-9-CLA concentrations, and to a greater extent *trans*-8,*cis*-10-CLA, *trans*-7,*cis*-9-CLA and *cis*-9,*trans*-11-CLA concentrations (Table 5). Inclusion of sunflower-seed

**Table 4.** Effect of experimental treatment on milk 18:1 composition (g/100 g total fatty acids) (Mean values with their standard errors for thirteen goats (experiment 1) and fourteen goats (experiment 2))

	Experiment 1				Experiment 2			
	H	HSO	HLO	SEM*	M	MSO	MLO	SEM*
<i>cis</i> -9-18:1	16.85 <sup>ac</sup>	20.63 <sup>b</sup>	17.99 <sup>a</sup>	0.717	13.70 <sup>a</sup>	15.69 <sup>b</sup>	15.25 <sup>b</sup>	0.349
<i>cis</i> -11-18:1	0.32 <sup>b</sup>	0.28 <sup>a</sup>	0.31 <sup>a,b</sup>	0.013	0.40 <sup>a</sup>	0.46 <sup>b</sup>	0.49 <sup>b</sup>	0.014
<i>cis</i> -12-18:1	0.14 <sup>a</sup>	0.64 <sup>c</sup>	0.34 <sup>b</sup>	0.033	0.15 <sup>a</sup>	0.79 <sup>c</sup>	0.60 <sup>b</sup>	0.067
<i>cis</i> -13-18:1	0.05 <sup>a</sup>	0.11 <sup>b</sup>	0.11 <sup>b</sup>	0.003	0.02 <sup>a</sup>	0.05 <sup>b</sup>	0.09 <sup>c</sup>	0.005
<i>cis</i> -15-18:1 †	0.15 <sup>a</sup>	0.21 <sup>b</sup>	0.32 <sup>c</sup>	0.010	0.05 <sup>a</sup>	0.16 <sup>a</sup>	0.84 <sup>b</sup>	0.099
<i>cis</i> -16-18:1	0.07 <sup>a</sup>	0.14 <sup>b</sup>	0.15 <sup>c</sup>	0.004	0.04 <sup>a</sup>	0.11 <sup>b</sup>	0.14 <sup>c</sup>	0.007
<i>trans</i> -4-18:1	tr <sup>a</sup>	0.03 <sup>a</sup>	0.02 <sup>b</sup>	0.001	tr <sup>a</sup>	0.03 <sup>b</sup>	0.02 <sup>a,b</sup>	0.003
<i>trans</i> -5-18:1	tr	0.03	0.02	0.002	0.01 <sup>a</sup>	0.04 <sup>b</sup>	0.02 <sup>a,b</sup>	0.004
<i>trans</i> -6,7,8-18:1	0.12 <sup>a</sup>	0.53 <sup>b</sup>	0.51 <sup>b</sup>	0.014	0.14 <sup>a</sup>	0.56 <sup>c</sup>	0.47 <sup>b</sup>	0.028
<i>trans</i> -9-18:1	0.19 <sup>a</sup>	0.59 <sup>c</sup>	0.54 <sup>b</sup>	0.013	0.21 <sup>a</sup>	0.58 <sup>c</sup>	0.47 <sup>b</sup>	0.011
<i>trans</i> -10-18:1	0.15	0.08	0.05	0.039	0.44 <sup>a</sup>	3.23 <sup>b</sup>	1.56 <sup>a</sup>	0.500
<i>trans</i> -11-18:1	1.51 <sup>a</sup>	9.02 <sup>c</sup>	8.14 <sup>b</sup>	0.362	1.17 <sup>a</sup>	8.50 <sup>c</sup>	5.36 <sup>b</sup>	0.465
<i>trans</i> -12-18:1	0.15 <sup>a</sup>	0.62 <sup>c</sup>	0.57 <sup>b</sup>	0.014	0.16 <sup>a</sup>	0.67 <sup>b</sup>	0.68 <sup>b</sup>	0.025
<i>trans</i> -13,14-18:1	0.22 <sup>a</sup>	0.59 <sup>b</sup>	0.91 <sup>c</sup>	0.046	0.29 <sup>a</sup>	0.87 <sup>b</sup>	1.66 <sup>c</sup>	0.096
<i>trans</i> -16-18:1 ‡	0.17 <sup>a</sup>	0.43 <sup>b</sup>	0.55 <sup>c</sup>	0.016	0.13 <sup>a</sup>	0.30 <sup>b</sup>	0.57 <sup>c</sup>	0.036

H, diet based on natural grassland hay (experiment 1) supplemented with no additional oil; HSO, diet based on natural grassland hay (experiment 1) supplemented with sunflower-seed oil; HLO, diet based on natural grassland hay (experiment 1) supplemented with linseed oil; M, diet based on maize silage (experiment 2) supplemented with no additional oil; MSO, diet based on maize silage (experiment 2) supplemented with sunflower-seed oil; MLO, diet based on maize silage (experiment 2) supplemented with linseed oil; tr, concentrations below 0.001 mg/100 g fatty acids.

<sup>a,b,c</sup> Mean values for each experiment within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* Error df 22 and 24 for experiments 1 and 2, respectively.

† Contains *trans*-17-18:1 as a minor component.

‡ Contains *cis*-14-18:1 as a minor component.

**Table 5.** Effect of experimental treatment on milk 18:2 composition (mg/100 g total fatty acids) (Mean values with their standard errors for thirteen goats (experiment 1) and fourteen goats (experiment 2))

	Experiment 1				Experiment 2			
	H	HSO	HLO	SEM*	M	MSO	MLO	SEM*
<i>cis</i> -9, <i>trans</i> -13-18:2	172 <sup>a</sup>	325 <sup>b</sup>	606 <sup>c</sup>	14.8	150 <sup>a</sup>	282 <sup>b</sup>	725 <sup>c</sup>	28.5
<i>cis</i> -9, <i>trans</i> -12-18:2	80 <sup>a</sup>	142 <sup>b</sup>	211 <sup>c</sup>	8.44	59 <sup>a</sup>	86 <sup>a</sup>	305 <sup>b</sup>	21.8
<i>trans</i> -9, <i>cis</i> -12-18:2	3 <sup>a</sup>	15 <sup>a</sup>	185 <sup>b</sup>	7.77	2 <sup>a</sup>	25 <sup>a</sup>	90 <sup>b</sup>	9.11
<i>trans</i> -9, <i>trans</i> -12-18:2	6 <sup>a</sup>	10 <sup>b</sup>	21 <sup>c</sup>	1.41	12 <sup>a</sup>	9 <sup>a</sup>	94 <sup>b</sup>	13.7
<i>trans</i> -9, <i>trans</i> -14-18:2	14 <sup>a</sup>	51 <sup>b</sup>	24 <sup>a</sup>	3.81	28 <sup>a</sup>	66 <sup>b</sup>	15 <sup>a</sup>	6.55
<i>trans</i> -11, <i>cis</i> -15-18:2	125 <sup>a</sup>	92 <sup>a</sup>	1485 <sup>b</sup>	54.3	73 <sup>a</sup>	135 <sup>a</sup>	2748 <sup>b</sup>	178.3
<i>trans</i> -11, <i>trans</i> -15-18:2	24 <sup>a</sup>	38 <sup>a</sup>	458 <sup>b</sup>	16.67	tr <sup>a</sup>	6 <sup>a</sup>	296 <sup>b</sup>	21.6
<i>cis</i> -9, <i>cis</i> -12-18:2	2129 <sup>b</sup>	2242 <sup>b</sup>	1381 <sup>a</sup>	61.5	2410 <sup>b</sup>	3013 <sup>c</sup>	1917 <sup>a</sup>	123.0
<i>cis</i> -9, <i>cis</i> -11-CLA	2 <sup>a</sup>	6 <sup>b</sup>	5 <sup>b</sup>	0.80	1 <sup>a</sup>	5 <sup>a,b</sup>	7 <sup>b</sup>	1.34
<i>cis</i> -9, <i>trans</i> -11-CLA	828 <sup>a</sup>	3694 <sup>b</sup>	3313 <sup>b</sup>	189.9	816 <sup>a</sup>	4266 <sup>c</sup>	2555 <sup>b</sup>	272.9
<i>cis</i> -12, <i>trans</i> -14-CLA	1 <sup>a</sup>	1 <sup>a</sup>	9 <sup>b</sup>	0.78	tr <sup>a</sup>	tr <sup>a</sup>	14 <sup>b</sup>	0.80
<i>trans</i> -7, <i>cis</i> -9-CLA	28 <sup>a</sup>	93 <sup>b</sup>	87 <sup>b</sup>	2.88	40 <sup>a</sup>	106 <sup>c</sup>	94 <sup>b</sup>	3.21
<i>trans</i> -8, <i>cis</i> -10-CLA	16 <sup>a</sup>	74 <sup>c</sup>	64 <sup>b</sup>	3.52	19 <sup>a</sup>	107 <sup>c</sup>	50 <sup>b</sup>	5.35
<i>trans</i> -9, <i>cis</i> -11-CLA	7 <sup>b</sup>	tr <sup>a</sup>	tr <sup>a</sup>	1.30	2 <sup>b</sup>	tr <sup>a</sup>	tr <sup>a</sup>	0.53
<i>trans</i> -10, <i>cis</i> -12-CLA	1 <sup>a</sup>	5 <sup>b</sup>	2 <sup>a</sup>	0.47	4 <sup>a</sup>	64 <sup>b</sup>	16 <sup>a</sup>	12.8
<i>trans</i> -11, <i>cis</i> -13-CLA	28 <sup>a</sup>	63 <sup>a</sup>	470 <sup>b</sup>	57.8	7 <sup>a</sup>	9 <sup>a</sup>	102 <sup>b</sup>	15.5
<i>trans</i> -12, <i>cis</i> -14-CLA	3 <sup>a</sup>	5 <sup>a</sup>	18 <sup>b</sup>	1.72	2 <sup>a</sup>	5 <sup>a</sup>	16 <sup>b</sup>	2.41
<i>trans</i> -7, <i>trans</i> -9-CLA	7 <sup>a</sup>	11 <sup>b</sup>	16 <sup>c</sup>	1.25	5 <sup>a</sup>	20 <sup>c</sup>	13 <sup>b</sup>	1.34
<i>trans</i> -8, <i>trans</i> -10-CLA	4 <sup>a</sup>	8 <sup>b</sup>	5 <sup>a</sup>	0.97	3 <sup>a</sup>	10 <sup>b</sup>	6 <sup>a,b</sup>	1.07
<i>trans</i> -9, <i>trans</i> -11-CLA	21 <sup>a</sup>	51 <sup>b</sup>	66 <sup>c</sup>	3.80	15 <sup>a</sup>	59 <sup>c</sup>	38 <sup>b</sup>	3.74
<i>trans</i> -10, <i>trans</i> -12-CLA	3 <sup>a</sup>	18 <sup>c</sup>	7 <sup>b</sup>	0.97	3 <sup>a</sup>	24 <sup>b</sup>	8 <sup>a</sup>	1.87
<i>trans</i> -11, <i>trans</i> -13-CLA	9 <sup>a</sup>	15 <sup>a</sup>	46 <sup>b</sup>	4.63	3 <sup>a</sup>	6 <sup>a</sup>	28 <sup>b</sup>	2.41
<i>trans</i> -12, <i>trans</i> -14-CLA	9 <sup>a</sup>	14 <sup>a</sup>	62 <sup>b</sup>	5.44	6 <sup>a</sup>	9 <sup>a</sup>	42 <sup>b</sup>	2.41

H, diet based on natural grassland hay (experiment 1) supplemented with no additional oil; HSO, diet based on natural grassland hay (experiment 1) supplemented with sunflower-seed oil; HLO, diet based on natural grassland hay (experiment 1) supplemented with linseed oil; M, diet based on maize silage (experiment 2) supplemented with no additional oil; MSO, diet based on maize silage (experiment 2) supplemented with sunflower-seed oil; MLO, diet based on maize silage (experiment 2) supplemented with linseed oil; tr, concentrations below 0.5 mg/100 g fatty acids; CLA, conjugated linoleic acid.

<sup>a,b,c</sup> Mean values for each experiment within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* Error df 22 and 24 for experiments 1 and 2, respectively.

oil in the diet resulted in a specific enrichment ( $P=0.001$ ) of *trans*-10,*trans*-12-CLA and *trans*-10,*cis*-12-CLA, while linseed oil enhanced ( $P<0.05$ ) milk fat *trans*-12,*trans*-14-CLA, *trans*-11,*trans*-13-CLA, *trans*-12,*cis*-14-CLA, *cis*-12,*trans*-14-CLA and *trans*-11,*cis*-13-CLA concentrations. In other respects, *trans*-12,*trans*-14-CLA, *trans*-11,*trans*-13-CLA, *cis*-12,*trans*-14-CLA and *trans*-11,*cis*-13-CLA concentrations were higher and *trans*-10,*cis*-12-CLA and *trans*-7,*cis*-9-CLA content was lower in milk from diets based on grassland hay compared with maize silage (Table 5).

#### Principal component analysis

PCA of data from both experiments allowed for the discrimination of three significant principal components (PC) that could account for proportionately 0.399 (PC1), 0.116 (PC2) and 0.109 (PC3) of the total variation in milk yield and composition, and milk fatty acid composition. From the biplot PC1  $\times$  PC2 resulting from PCA applied to different dietary treatments, four clusters were apparent, with PC1 discriminating on the basis of the lipid supplementation and PC2 discriminating the two more extreme diets (HLO and MSO) (Fig. 1 (a)). Similarly, the biplot PC1  $\times$  PC3 revealed two clusters that PC3 discriminated on the basis of plant oil composition (linseed oil *v.* sunflower-seed oil) (Fig. 1 (b)).

On the basis of the PC1  $\times$  PC2 loading plot that accounted for proportionately 0.515 of total variation in milk yield, composition and fatty acid concentration, four clusters of specific fatty acids with a correlation coefficient with PC of  $>0.6$  or  $<-0.6$  were distinguished. The PC1 (Fig. 1 (c)) contains two groups of fatty acids with different metabolic origins. Fatty acids synthesised *de novo* (10:0, 12:0, 14:0, 16:0), medium-chain  $\Delta$ -9 desaturase products (*cis*-9-10:1, *cis*-9-12:0, *cis*-9-14:1, *cis*-9-16:1) and odd- and branched-chain fatty acids (13:0, *iso*-14, *iso*-16, 15:0, *anteiso*-15 and 17:0) were clustered as a single group that was negatively correlated with PC1. In contrast, long-chain SFA (18:0), MUFA (*trans*-9-16:1, *cis*-12-18:1, *cis*-13-18:1, *cis*-16-18:1, *trans*- $\Delta^{6-12,16}$ -18:1), CLA (*trans*-9,*trans*-11-CLA, *trans*-8,*cis*-10-CLA) and long-chain  $\Delta$ -9 desaturase products (*trans*-7,*cis*-9-CLA, *cis*-9,*trans*-11-CLA and *cis*-9,*trans*-13-18:2) were clustered in a second group that was positively correlated to PC1. The PC2 (Fig. 1 (c)) distinguished between 18:2*n*-6 and 18:3*n*-3 in the diet, with correlation coefficients with PC2 of 0.60 and  $-0.84$ , respectively. PC3 (data not shown) discriminated on the basis of plant oil composition with positive and negative relationships observed for sunflower-seed oil- and linseed oil-supplemented diets, respectively. On the basis of correlation coefficients with PC3, two clusters of fatty acids were identified containing *iso*-17:0 ( $r > 0.6$ ) in one group and 6:0, 8:0, *trans*-11,*cis*-15-18:2 and *cis*-12,*trans*-14-CLA in the other ( $r < -0.6$ ).

#### Discussion

Unique features of the present study included a comprehensive determination of milk fatty acid composition responses to plant oils in goats fed diets containing grassland hay or maize silage. Detailed measurements of milk fat composition are important in understanding the potential impact of modified goat-derived foods on human-related outcomes as

well as providing a basis for between-species comparisons with data derived from published studies in lactating cows fed comparable diets. Furthermore, characterisation of specific effects on *trans*-18:1, 18:2 and CLA isomers also provides further insight into the possible role of biohydrogenation intermediates in the regulation of milk fat synthesis in the goat compared with the known anti-lipogenic activity of fatty acid intermediates in the lactating cow.

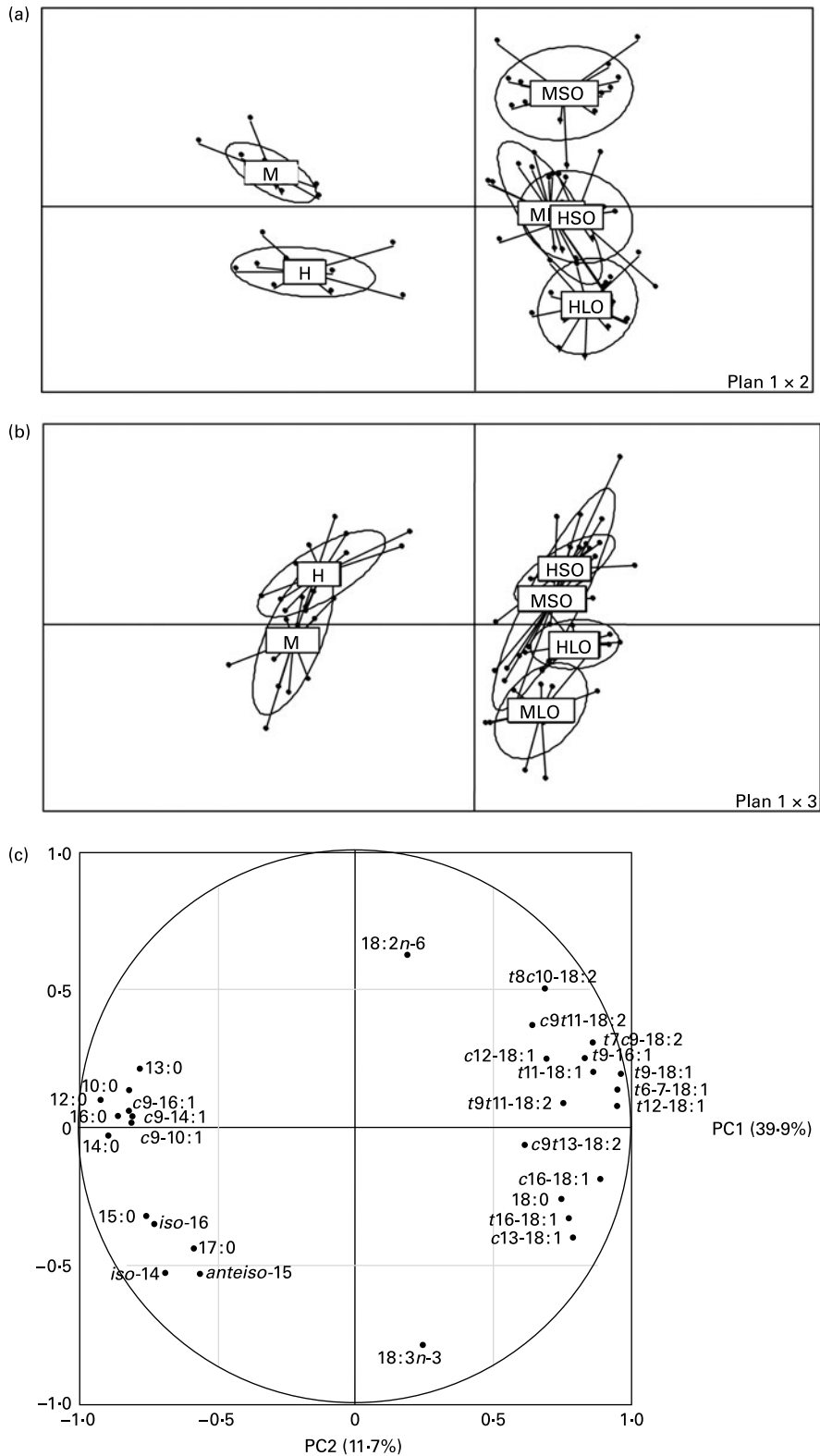
The effect of forage type on milk fatty acid composition responses to plant oils was assessed in two independent experiments at the same time of the year rather than in a single study. Even though all experimental treatments were not evaluated simultaneously, strong inferences on the role of forage in the diet can be drawn since the milk production potential, parity, stage of lactation and genotype at the  $\alpha S1$  casein locus of experimental animals were similar across experiments, and the fatty acid composition of plant oils was comparable between experiments (Table 1). Furthermore, data were also analysed by PCA, with between-experiment differences in responses to lipid supplements being interpreted as attributable, at least in the most part, to the composition of the basal diet.

#### Animal performance

Milk production and composition responses to sunflower-seed oil and linseed oil were consistent with previous studies<sup>(9)</sup>, supporting the view that plant oils typically have no effect on milk yield, enhance milk fat secretion, but induce variable effects on milk protein concentrations in goats. In contrast, supplements of oils rich in PUFA often result in diet-induced milk fat depression in lactating cows<sup>(13,17,30)</sup>. However, inclusion of sunflower-seed oil in maize silage-based diets increased milk yield, but had no effect on milk fat content, highlighting the important role of the basal diet composition on milk production responses to lipid supplements in goats. Increases in milk fat content (5.6 and 5.1 g/kg, respectively) to the HSO and HLO treatments are in line with increases of 6.6 or 7.2 g/kg reported for goats fed diets based on cocksfoot hay supplemented with formaldehyde-treated linseed<sup>(31)</sup> or linseed oil<sup>(13)</sup>.

#### Milk fatty acid composition

**Effect of plant oils.** The impact of linseed oil and sunflower-seed oil on the concentrations of major fatty acids in milk were consistent between experiments (Fig. 1(a) and (c)) and in general agreement with earlier studies in lactating cows and goats<sup>(5,7)</sup>. Irrespective of forage type, plant oils in the diet decreased the concentration of milk fatty acids (10:0, 12:0, 14:0 and 16:0) synthesised *de novo* (Table 3), consistent with previous studies in lactating goats<sup>(5,31)</sup> and cows<sup>(5,7)</sup>. Reductions in the concentration of fatty acids derived from *de novo* synthesis were accompanied by decreases in milk odd- and branched-chain fatty acids other than *iso*-17:0 content, consistent with the changes determined in lactating cows<sup>(26,32,33)</sup>. Overall, the changes in odd- and branched-chain fatty acids and 10:0–16:0 concentrations (Fig. 1 (c)) suggest that the incorporation of both groups of fatty acids into milk share a common point of regulation which is inhibited by 18:2*n*-6 or 18:3*n*-3 and/or fatty acid intermediates formed during ruminal PUFA metabolism.



**Fig. 1.** Principal component analysis of data derived from the analysis of eighty-one milk samples. (a) Distribution of samples based on the first two principal components (PC1 and PC2). Each group represents a dietary treatment and each point is the barycentre of data for individual goats. (b) Distribution of samples based on the primary and tertiary principal components (PC1 and PC3). (c) Plot of experimental variables projected on the basis of the first two principal components (PC1 and PC2) that describe the association between milk yield, milk composition and milk fatty acids concentration. Only measured parameters with correlation coefficients ( $< -0.60$  or  $> 0.60$ ) for a single principal component are indicated. H, diet based on natural grassland hay (experiment 1) supplemented with no additional oil; HSO, diet based on natural grassland hay (experiment 1) supplemented with sunflower-seed oil; HLO, diet based on natural grassland hay (experiment 1) supplemented with linseed oil; M, diet based on maize silage (experiment 2) supplemented with no additional oil; MSO, diet based on maize silage (experiment 2) supplemented with sunflower-seed oil; MLO, diet based on maize silage (experiment 2) supplemented with linseed oil; *c*, *cis*; *t*, *trans*.



Plant oils enhanced milk fat 18:0, *cis*-12-18:1, *trans*-18:1 ( $\Delta^{6-9,11-14,16}$ ), 18:2  $\Delta$ -9 desaturase products (*cis*-9,*trans*-13-18:2, *cis*-9,*trans*-11-CLA, *trans*-7,*cis*-9-CLA), *trans*-7, *trans*-9-CLA, *trans*-9,*trans*-11-CLA and *trans*-8,*cis*-10-CLA concentrations. Earlier studies have also shown that lipids rich in 18:2 $n$ -6 or 18:3 $n$ -3 increase one or more of these fatty acids in bovine or caprine milk<sup>(5,7)</sup>.

Inclusion of lipids rich in 18:2 $n$ -6 and 18:3 $n$ -3 resulted in substantial increases in milk *cis*-9,*trans*-11-CLA and *trans*-11-18:1 concentrations that were closely associated across experiments ( $y = 0.437x + 0.133$ ;  $n$  81;  $r + 0.92$ ;  $P < 0.001$ ; Fig. 1 (c)), confirming the product–precursor relationship for  $\Delta$ -9 desaturase established in bovine<sup>(17)</sup> and caprine<sup>(9)</sup> milk fat. This close relationship also suggests that larger proportionate increases in *trans*-11-18:1 supply at the mammary gland account for the larger milk fat *cis*-9,*trans*-11-CLA responses to plant oils on maize silage-based diets in the goat<sup>(2,5)</sup> compared with the cow<sup>(19)</sup>. Furthermore, increases in milk fat *cis*-9,*trans*-11-CLA content to plant oils have been shown to persist over a 10-week period in goats fed diets based on hay, concentrates or maize silage<sup>(5)</sup>, whereas responses are transient and decrease over time in cows fed high-concentrate or maize silage-based diets<sup>(18,19)</sup>.

Both plant oils in the diet enhanced milk *cis*-9-18:1 content on maize silage-based diets, whereas only sunflower-seed oil enhanced *cis*-9-18:1 in milk on grassland hay-based diets. A large proportion of *cis*-9-18:1 secreted in milk is derived via the action of  $\Delta$ -9 desaturase on 18:0<sup>(15,17)</sup>, suggesting that most of the increases in *cis*-9-18:1 to plant oils can be attributed to increases in ruminal 18:0 outflow. However, the relative increases in *cis*-9-18:1 concentrations in both experiments are lower than could be expected when comparable diets are fed to lactating cows<sup>(5,10,19)</sup>. Inter-species differences may be related to the relative sensitivity of the  $\Delta$ -9 desaturase enzyme system to the inhibitory effects of PUFA which appear to be greater in the goat than the cow<sup>(34)</sup> and/or to larger increases in the enrichment of *trans*-9,*trans*-11-CLA in milk (that is a known inhibitor of bovine  $\Delta$ -9 desaturase<sup>(35)</sup>) in the goat determined in the present study compared with cows receiving diets comparable with the MSO and HLO treatments<sup>(19)</sup>.

**Specific effects of sunflower-seed oil.** Amongst the six diets evaluated in the present study, two treatments were discriminated by PCA: MSO and HLO (Fig. 1 (a)). This suggests that interactions between carbohydrate composition and PUFA in the diet are an important determinant of the extent of ruminal biohydrogenation, formation of specific biohydrogenation intermediate products and mammary metabolism in the goat, factors that have been shown to have a role in the regulation of milk fat composition in lactating cows<sup>(5,7,36)</sup>.

Increases in milk fat *trans*-8,*cis*-10-CLA and *trans*-10,*trans*-12-CLA concentrations were higher in response to sunflower-seed oil than linseed oil. Comparisons between-experiments also indicated that the increases in these CLA isomers were greater for maize silage than grassland hay-based diets (Fig. 1 (c)). Previous studies have shown that 18:2 $n$ -6 increases ruminal outflow of *trans*-10,*trans*-12-CLA<sup>(37)</sup> and that plant oils rich in 18:2 $n$ -6 enhance concentrations of *trans*-10,*trans*-12-CLA, *trans*-8,*cis*-10-CLA and *trans*-7,*cis*-9-CLA in bovine milk<sup>(19,32)</sup>.

Treatment MSO resulted in the highest milk fat *trans*-10-18:1 concentration, consistent with earlier comparisons of

milk fat responses to dietary unsaturated fatty acids in goats fed maize silage- or lucerne hay-based diets<sup>(5,13)</sup>. The extent of unsaturated fatty acid metabolism in the rumen and the formation of specific intermediates in the cow is known to be dependent on diet composition, with the flow of *trans*-10-18:1 at the duodenum tending to replace *trans*-11-18:1 when the ratio of starch:fibre in the diet is increased<sup>(17,30)</sup>. However, the concentration of *trans*-10-18:1 in milk from goats fed MSO (3.2 g/100 g fatty acids) was lower than in milk from cows offered a similar diet (corresponding concentration 7.2)<sup>(19)</sup>, while the reverse was true for *trans*-11-18:1 (8.5 v. 1.4 g/100 g fatty acids). These findings point towards the major pathways of biohydrogenation of unsaturated fatty acids in goats being less influenced by alterations in diet composition compared with the cow, which may be related to a higher rate of salivary secretion<sup>(38)</sup> and associated larger buffering capacity, resulting in a more stable rumen pH in goats compared with cows. Direct comparisons of ruminant species fed the same diet would be required to confirm these considerations.

Concentrations of *trans*-10,*cis*-12-CLA in milk were enhanced in response to sunflower-seed oil, but the increases were lower for diets based on grassland hay than maize silage. Furthermore, *trans*-10,*cis*-12-CLA content in milk from treatment MSO (0.064 g/100 g fatty acids) was higher compared with milk fat from cows fed a similar diet (<0.03 g/100 g fatty acids)<sup>(19)</sup>. Across experiments, milk fat *trans*-10-18:1 content was closely associated with *trans*-10,*cis*-12-CLA concentrations ( $y = 41.2x + 0.465$ ;  $n$  81;  $r + 0.93$ ;  $P < 0.001$ ), suggesting that both biohydrogenation intermediates are formed during 18:2 $n$ -6 metabolism in the rumen, consistent with earlier considerations on the possible role of ruminal biohydrogenation on the regulation of milk fat synthesis<sup>(17)</sup> and more recent data examining the formation of biohydrogenation intermediates on 18:2 $n$ -6-rich diets<sup>(37)</sup>. However, the slope of the putative precursor–product in this experiment appears lower compared with that between *trans*-10-18:1 and *trans*-10,*cis*-12-CLA in bovine milk ( $y = 356x - 0.41$ ;  $r + 0.95$ ;  $P < 0.05$ )<sup>(19)</sup>. This tends to suggest that reduction of biohydrogenation intermediates in the rumen is less extensive in the goat relative to the cow, but direct comparisons of ruminal lipid metabolism in the goat and cow are required to confirm possible between-species differences. Concentrations of *trans*-10-18:1 and *trans*-10,*cis*-12-CLA were also significantly associated with *iso*-17:0 in milk ( $r$  values 0.72), in direct contrast with measurements reported for studies in cows<sup>(11)</sup>. *Iso*-17:0 is mainly synthesised by rumen bacteria which exhibit large differences in *iso*-17:0 depending on microbial species<sup>(39)</sup>. It appears plausible that the relative abundance and proliferation of specific rumen bacterial populations in response to changes in nutrient supply may also differ between ruminant species.

In contrast to cows, plant oils in the diet increase milk fat content and yield in goats<sup>(5,9)</sup>. However, milk fat synthesis was not increased on the MSO treatment which may be related to an increase in ruminal formation of biohydrogenation intermediates that limit lipogenic effects of plant lipids in goats, consistent with an increase in milk fat *trans*-10,*cis*-12-CLA content. However, post-ruminal infusion studies have provided evidence that the goat is not as responsive to the anti-lipogenic effects of *trans*-10,*cis*-12-CLA<sup>(40)</sup> compared

with the cow<sup>(41,42)</sup>. In addition to *trans*-10,*cis*-12-CLA<sup>(17,43)</sup>, post-ruminal infusions in lactating cows have provided evidence that *cis*-10,*trans*-12-CLA<sup>(44)</sup> and *trans*-9,*cis*-11-CLA<sup>(35)</sup> may also exert anti-lipogenic effects and decrease milk fat synthesis. However, these CLA isomers remained very low in goats whatever the dietary treatments in the present study. Differences in milk fat synthesis responses between the goat and cow to changes in diet composition and post-ruminal *trans*-10,*cis*-12-CLA infusions suggest species-specific points of regulation of both ruminal and mammary metabolism.

**Specific effects of linseed oil.** For both experiments, linseed oil resulted in higher enrichment of *trans*-13,14-18:1, *trans*-16-18:1, *cis*-15-18:1, *cis*-16-18:1 and *cis*-9,*trans*-13-18:2 in milk fat relative to diets containing sunflower-seed oil. Inclusion of linseed oil in grassland hay- or maize silage-based diets resulted in specific increases in milk fat *trans*-11,*trans*-13-CLA, *trans*-12,*trans*-14-CLA, *trans*-12, *cis*-14-CLA, *cis*-12,*trans*-14-CLA and *trans*-11,*cis*-13-CLA concentrations, which is in line with the studies examining the effects of diet on the CLA isomer distribution in bovine milk fat<sup>(19,32)</sup>. Furthermore, linseed oil in the diet in both experiments enhanced ( $P < 0.001$ ) milk fat *cis*-9,*trans*-12-18:2, *cis*-9,*trans*-13-18:2, *trans*-9,*trans*-12-18:2, *trans*-11,*trans*-15-18:2, *trans*-9,*cis*-12-18:2 and *trans*-11, *cis*-15-18:2 concentrations, consistent with the changes reported for bovine milk to diets containing linseed oil or linseeds<sup>(7)</sup>, providing further support that specific non-methylene-interrupted 18:2 isomers are derived from ruminal 18:3*n*-3 metabolism. Irrespective of forage source, *cis*-9,*trans*-13-18:2 concentrations were higher on linseed oil (+252 and +383 % for H and M diets, respectively) than sunflower-seed oil (+89 and +88 % for H and M diets, respectively) supplemented diets. This suggests that, in goats as in cows<sup>(19)</sup>, *cis*-9,*trans*-13-18:2 is derived from the action of  $\Delta 9$ -desaturase in the mammary gland on *trans*-13-18:1 formed during 18:3*n*-3 metabolism in the rumen<sup>(7,45,46)</sup>. The present data also indicated that linseed oil in the diet decreases milk 18:2*n*-6 content, which is in agreement with observations in lactating cows<sup>(5)</sup>.

Concentrations of *trans*-11,*cis*-13-CLA were increased by linseed oil in the diet, responses that were higher on hay- than maize silage-based diets and represented the second most abundant CLA isomer, consistent with previous determinations of bovine milk from diets rich in 18:3*n*-3<sup>(19,32,47)</sup>. Indirect comparisons indicated a higher *trans*-11,*cis*-13-CLA concentration in caprine milk on the HLO treatment (0.47 g/100 g fatty acids) compared with milk from cows fed a similar diet (0.20 g/100 g fatty acids)<sup>(19)</sup>. Increases in milk fat 18:3*n*-3 content were confined to the inclusion of linseed oil on maize silage-based diets. These findings would appear to support reduced ruminal 18:3*n*-3 biohydrogenation on diets containing a higher proportion of starch and relatively low amounts of neutral-detergent fibre<sup>(36)</sup>.

### Conclusions

Plant oils in the diet enhanced milk fat synthesis in lactating goats and altered milk fatty acid composition. Changes in milk fatty acid composition were dependent on forage type and plant oil composition, with evidence of an interaction

between these nutritional factors. Responses to lipid supplements were characterised as a reduction in fatty acids synthesised *de novo* (10:0–16:0) and an increase in 18:0, *cis*-18:1, CLA and PUFA concentrations, indicating that plant oils can be used to effect potentially beneficial changes in milk fat composition without inducing detrimental effects on animal performance. Indirect comparisons with published data in cows point towards species differences in the response to dietary lipid supplements that include: (i) a lower propensity for alterations in ruminal biohydrogenation towards *trans*-10-18:1 at the expense of *trans*-11-18:1 in the goat compared with the cow; (ii) no significant occurrence of *trans*-9,*cis*-11-CLA and a higher incorporation of *trans*-9,*trans*-11-CLA in caprine relative to bovine milk fat; (iii) a lower inhibition of *de novo* fatty acid synthesis by PUFA in the diet or intermediates formed during ruminal metabolism in the goat relative to the cow. Further research is required to establish the causal mechanisms accounting for inter-species variation in lipogenic responses to dietary plant oil supplements.

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