British Journal of Nutrition (2009), **101**, 1006–1019
doi:10.1017/S0007114508051696

Effect of calcium salts of a mixture of conjugated linoleic acids containing *trans*-10, *cis*-12 in the diet on milk fat synthesis in goats

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(Received 7 April 2008 – Revised 15 July 2008 – Accepted 24 July 2008 – First published online 25 September 2008)

Dietary supplements of conjugated linoleic acid (CLA) containing *trans*-10, *cis*-12 CLA decrease milk fat secretion in the lactating cow and sheep, but their effects on mammary lipogenesis in the goat are less well defined. Eight lactating goats were used in two 4 × 4 Latin-square experiments with 14 d experimental periods to examine the effects of calcium salts of CLA methyl esters (CaCLA) containing *trans*-10, *cis*-12 on milk fat synthesis. Experimental treatments consisted of incremental inclusion of 0, 30, 60 or 90 g of CaCLA/d (corresponding to 7·47, 14·9 and 22·4 g/d of *trans*-10, *cis*-12 CLA) offered during the first 10 d of each experimental period that replaced maize grain in concentrates (Experiment 1) or calcium salts of palm oil fatty acids (Experiment 2). Relative to the control, inclusion of 30, 60 or 90 g CaCLA/d in the diet reduced milk fat yield by 19·8, 27·9 and 32·3 % and 17·5, 39·0 and 49·3 % in Experiments 1 and 2, respectively. Decreases in milk fat were due to reductions in the secretion of fatty acids synthesised *de novo* rather than the uptake of fatty acids from the peripheral circulation. Indirect comparisons with the studies in the lactating cow indicated a lower efficacy of CaCLA supplements on mammary lipogenesis in the goat. In conclusion, CaCLA in the diet inhibits milk fat synthesis in the goat, responses that are dependent on the supply of dietary fatty acids, with evidence that the caprine is less sensitive to the anti-lipogenic effects of *trans*-10, *cis*-12 CLA compared with the bovine or ovine.

Conjugated linoleic acid: Milk fat synthesis: Goats: Lactation

It is well established that *trans*-10, *cis*-12 conjugated linoleic acid (CLA) is involved in the regulation of lipid metabolism in a number of mammalian species including the pig, cow and human[1]. Numerous studies have characterised the anti-lipogenic effects of *trans*-10, *cis*-12 CLA on milk fat synthesis in the lactating cow. In the bovine, reductions in milk fat are known to occur in a predictable and dose-dependent manner in response to post-ruminal infusions[2–4] or rumen-protected supplements of *trans*-10, *cis*-12 CLA[5–7]. While the anti-lipogenic effects are well characterised in the lactating cow, the role of *trans*-10, *cis*-12 CLA in lipogenesis in other lactating ruminant species is less well defined.

The effects of stage of lactation on milk fat are known to be comparable in the bovine and caprine, but changes in milk fat synthesis to lipid supplements in the diet differ markedly between these species[8,9]. Typically, milk fat content is increased in response to dietary fat in the goat, but not in the cow, which may reflect inter-species differences in ruminal lipid metabolism and/or the regulation of cellular processes in the mammary gland and the relative importance of key enzymes in the synthesis of milk fatty acids[8,9]. Recent studies examining short-term intravenous[10] or post-ruminal infusions[11] of *trans*-10, *cis*-12 CLA in goats have reported relatively minor or no effects on mammary lipogenesis. However, rumen-protected supplements of *trans*-10, *cis*-12 CLA were reported to decrease milk fat synthesis in goats[12,13], but the quantity required to inhibit milk fat synthesis in the goat was much higher than expected based on metabolic live-weight comparisons with the lactating cow. While inconclusive, the available evidence points towards a lower sensitivity to the anti-lipogenic effects of *trans*-10, *cis*-12 CLA in the caprine than in the bovine.

Further studies have demonstrated that the anti-lipogenic effects of *trans*-10, *cis*-12 CLA on milk fat synthesis in the lactating cow are markedly reduced when diets contain relatively high amounts of rumen-protected unsaturated fatty acids[14]. Inter-species differences in milk fat responses to lipid supplements[8,9] also suggest that the supply of fatty acid precursors available to the mammary gland would have a more critical role on the inhibitory effects of *trans*-10, *cis*-12 CLA on milk fat synthesis in the goat compared with the cow.

Eight lactating alpine goats were used in two 4 × 4 Latin-square experiments to examine milk fat responses to incremental inclusion of calcium salts of a mixture of CLA isomers (CaCLA) containing *trans*-10, *cis*-12 in the diet. In order to establish the possible role of long-chain fatty acid precursor supply, CaCLA replaced maize grain in concentrate supplements (Experiment 1) or calcium salts of palm oil fatty acids (Experiment 2) in the diet. For both experiments, milk composition responses to the same range in *trans*-10, *cis*-12 CLA intakes were evaluated. CaCLA were selected as a source of rumen-protected *trans*-10, *cis*-12 CLA since the

Abbreviations: CaCLA, calcium salts of CLA methyl esters; CLA, conjugated linoleic acid; FAME, fatty acid methyl esters; CaPO, salts of palm oil fatty acid.

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effects of these lipid supplements on milk fat synthesis have been determined in short-term experiments[5,15] and extensively evaluated over an extended period during early[6,7,16] and established lactation in the cow[17,18], allowing an indirect comparison of the anti-lipogenic effects of *trans*-10, *cis*-12 CLA between ruminant species.

Materials and methods

Animal management and experimental design

All experimental procedures were approved by the Institut National de la Recherche Agronomique Animal Care and Use Committee in accordance with the guidelines on the use of animals for experimental purposes implemented in France[19]. Eight lactating non-pregnant alpine goats in mid-lactation of mean 250 (SE 8·3) d in lactation, 63·9 (SE 4·33) kg live weight and producing 2·58 (SE 0·121) kg milk/d were used in two experiments each conducted as a 4 × 4 Latin square with 14 d experimental periods. Animals were assigned to Latin squares according to milk yield and milk composition determined over a 4 d period immediately before the start of the experiment. Mean milk yield (kg/d), milk fat, protein and lactose content (g/kg) were 2·59 (SE 0·085), 42·0 (SE 1·30), 39·6 (SE 1·20) and 43·9 (SE 1·65), and 2·58 (SE 0·235), 39·6 (SE 1·35), 36·7 (SE 0·40) and 42·9 (SE 1·05) for the goats used in Experiments 1 and 2, respectively. Experimental animals were housed in individual stalls and had continuous access to water. Daily rations were fed equal meals at 08.30 and 16.00 hours and goats were milked at 06.00 and 15.30 hours.

Experimental treatments consisted of CaCLA prepared from chemical isomerisation of sunflower-seed oil and containing *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA as major components (Im'prouve ALC, Xeris S.A., Séné, France; Table 1), and included in the diet at a rate of 0, 30, 60 or 90 g/d corresponding to 7·47, 14·9 and 22·4 g of *trans*-10, *cis*-12 CLA/d. Supplements of CaCLA were fed for the first 10 d of each experimental period but removed from daily rations during the remaining 4 d in order to minimise treatment carry-over effects. In Experiment 1, CaCLA replaced maize grain in concentrate supplements (treatments C0, C1, C2 and C3, respectively), while in Experiment 2 CaCLA substituted for calcium salts of palm oil fatty acids (CaPO) (treatments CP0, CP1, CP2 and CP3; Ruminer, Aurillac, France; Table 1, respectively). Replacing maize grain with CaCLA supplements also enhanced diet energy content in Experiment 1, whereas treatments CP0, CP1, CP2 and CP3 in Experiment 2 were formulated to be isoenergetic.

Experimental diets

Goats were offered lucerne hay *ad libitum* supplemented with 0·65 kg DM/d concentrates of variable composition (Table 2). Diets were formulated to supply 110 and 130 % of predicted energy and protein requirements, respectively[20]. In Experiment 1, CaCLA replaced (on a DM basis) maize grain in concentrate supplements or substituted for calcium salts of palm oil fatty acids in Experiment 2 (Table 2). For both experiments, concentrate supplements were fed at a fixed rate to avoid possible selection of dietary components, maintain the forage:concentrate ratio of the diet and ensure that experimental treatments supplied the targeted amount of *trans*-10, *cis*-12 CLA.

Table 1. Fatty acid composition of lipid supplements

	Supplement	
Fatty acid composition (g/100 g fatty acids)	CaPO	CaCLA
10 : 0	0·05	0·00
12 : 0	0·41	0·00
14 : 0	1·62	0·19
15 : 0	0·00	0·00
16 : 0	50·58	8·72
cis-9-16 : 1	0·18	0·11
17 : 0	0·10	0·00
18 : 0	3·70	3·21
cis-9-18 : 1	33·62	12·24
cis-11-18 : 1	0·00	0·88
18 : 2*n*-6	8·35	0·73
cis-9,*cis*-11 CLA	0·00	1·08
cis-10,*cis*-12 CLA	0·00	0·89
cis-9,*trans*-11 CLA	0·00	33·33
cis-11,*trans*-13 CLA	0·00	0·14
trans-7,*cis*-9 CLA	0·00	0·07
trans-8,*cis*-10 CLA	0·00	1·51
trans-10,*cis*-12 CLA	0·00	31·08
trans-7,*trans*-9 CLA	0·00	0·07
trans-8,*trans*-10 CLA	0·00	0·31
trans-9,*trans*-11 CLA	0·00	1·09
trans-10,*trans*-12 CLA	0·00	1·10
trans-11,*trans*-13 CLA	0·00	0·06
trans-12,*trans*-14 CLA	0·00	0·01
Σ CLA	0·00	72·03
18 : 3*n*-3	0·24	0·00
20 : 0	0·19	0·00
22 : 0	0·00	0·30
Unidentified	1·00	0·46
Σ Fatty acids (g/kg DM)	884	770

CaPO, calcium salts of palm oil fatty acids; CaCLA, calcium salts of conjugated linoleic acid.

Experimental measurements and sampling

Foods

Individual intakes were recorded daily but only measurements made on days 9 and 10 of each experimental period were used for statistical analysis. During this interval, samples of lucerne hay, concentrate mixtures C, CP, CP1 and CP2, calcium salts of palm oil fatty acids and CaCLA supplements were collected, composited and stored at −20°C. Feed DM content was determined after drying at 105°C for 24 h. Samples of maize silage, lucerne hay and concentrates were dried at 48°C for 48 h, passed through a 1 mm screen and submitted for the determination of chemical composition according to standard procedures[21]. Additional samples of experimental feeds were collected, lyophilised (Thermovac TM-20, Froilabo, Ozoir-la-Ferrière, France) and submitted for fatty acid determinations.

Milk

Milk yields were recorded daily, but only measurements on days 9 and 10 of each experimental period were analysed statistically. Samples of milk were collected from individual goats over four consecutive milkings starting 06.00 hours on day 9

Table 2. Ingredient and chemical composition of experimental hay and concentrate supplements

		Concentrate*				
	Hay	C	CP0	CP1	CP2	CP3
Ingredient (g/kg DM)†						
Maize grain		561	468	487	520	561
Maize silage		135	124	130	134	135
Dehydrated sugarbeet pulp		143	131	137	141	143
Soyabean meal		144	132	138	142	144
CaPO‡		0	130	91	47	0
Minerals and vitamins§		16	15	16	16	16
Composition‖						
DM (g/kg)	837	790	810	802	796	790
OM	903	949	938	941	945	949
CP	177	145	129	135	140	145
NDF	567	219	195	204	212	219
ADF	389	120	108	113	117	120
Starch	0	422	355	370	393	422
12 : 0	0·09	0·01	0·47	0·33	0·18	0·01
14 : 0	0·14	0·03	1·84	1·30	0·68	0·03
16 : 0	2·37	3·77	60·2	43·3	24·0	3·77
cis-9-16 : 1	0·02	0·04	0·23	0·17	0·11	0·04
18 : 0	0·33	0·51	4·60	3·38	1·97	0·51
cis-9-18 : 1	0·17	6·20	43·1	32·1	19·4	6·20
cis-11-18 : 1	0·03	0·18	0·15	0·16	0·17	0·18
18 : 2*n*-6	1·25	11·9	19·6	17·2	14·6	11·91
18 : 3*n*-3	1·82	0·74	0·93	0·88	0·81	0·74
20 : 0	0·07	0·08	0·28	0·22	0·15	0·08
22 : 0	0·08	0·04	0·04	0·04	0·04	0·05
24 : 0	0·11	0·06	0·06	0·06	0·06	0·06
Other fatty acids	0·84	0·50	1·72	1·35	0·94	0·50
Σ Fatty acids	7·30	24·1	133	100	63·1	24·1

ADF, acid-detergent fibre; CP, crude protein; NDF, neutral-detergent fibre; OM, organic matter; CaPO, calcium salts of palm oil fatty acids.

* A control concentrate supplement fed in experiment 1 (C) or concentrates fed in experiment 2 containing CaPO that were replaced incrementally with 0, 49, 83 or 130 g/kg diet DM of calcium salts of conjugated linoleic acid (CP0, CP1, CP2 and CP3, respectively).

† Declared ingredient and chemical composition do not include supplements of calcium salts of conjugated linoleic acid.

‡ Calcium salts of palm oil fatty acids.

§ Proprietary mineral and vitamin supplement (Centraliment, Aurillac, France) declared as containing (g/kg) sodium (50), calcium (200), phosphorus (45), magnesium (45), zinc (6) and manganese (3·5); (mg/kg) retinyl acetate (206), cholecalciferol (3·0) and DL-tocopheryl acetate (1300).

‖ g/kg DM, unless otherwise stated.

of each experimental period, preserved with potassium dichromate (Merck, Fontenay-Sous-Bois, France) and stored at 4°C until analysed for fat, crude protein and lactose content. Milk fat, crude protein and lactose were determined by near IR spectroscopy[21] calibrated using reference caprine milk samples. Unpreserved milk samples were also collected at 15.30 hours on day 9 and 06.00 hours on day 10, stored at −20 °C, composited according to yield and submitted for fatty acid analysis.

Live weight

Goats were weighed at the beginning of the experiment and on the last day of each experimental period at 11.00 hours.

Fatty acid analysis

Lipid in lucerne hay, concentrate ingredients and CaCLA supplements was extracted[22] and transesterified to fatty acid methyl esters (FAME) by incubation with methanolic hydrochloric acid according to standard procedures[23] using 23:0 (Sigma, St-Quentin Fallavier, France) as an internal standard. Lipid content and fatty acid composition of CaPO supplements were determined using the same extraction procedure[22], with the exception that the filtration step was omitted according to the recommendations of the manufacturer and the organic extract was transesterified to FAME using methanolic hydrochloric acid as a catalyst[23].

For milk fatty acid determinations, lipid in 1 ml samples was extracted and transesterified to FAME using freshly prepared methanolic sodium methoxide[24,25]. 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 a fused silica capillary column (100 m × 0·25 mm internal diameter) coated with 0·2 μm film of cyanopropyl polysiloxane (CP-SIL 88; Chrompack 7489, Middelburg, The Netherlands) using hydrogen as the carrier gas operated at constant pressure (125 kPa). Total FAME profile in a 2 μl sample at a split ratio of 1:40 was determined using a temperature gradient programme[25]. Injector and detector temperatures were maintained at 255 and 260°C, respectively. Peaks were routinely identified by comparison of retention times with FAME standards (GLC 463, Nu Chek Prep Inc., Elysian, MN, USA; reference mixture 47 885, Supelco, Bellefonte, PA, USA) 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[26]. Methyl esters not contained in commercially available standards were identified based on the comparisons with reference milk fat samples of known fatty acid composition based on the GC–MS analysis of 4,4-dimethyloxazoline fatty acid derivatives[27,28].

Following GLC analysis, samples of milk fat FAME were evaporated under nitrogen, dissolved in heptane and the distribution of CLA isomers 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, UK) coupled in series and 0·1 % (v/v) acetonitrile in heptane as the mobile phase[29]. 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[30]. Identification was verified by cross-referencing with the elution order reported in the literature[31] using *cis*-9, *trans*-11 CLA as a landmark isomer.

Statistical analysis

Experimental data were subjected to ANOVA using the general linear model procedure of Statistical Analysis Systems software package version 9.1 (SAS Institute, Inc., Cary, NC, USA) with a model that included the random effects of the goat and fixed effects of period and treatment. Sums of squares for treatment effects were further separated using orthogonal contrasts into single-degree-of-freedom comparisons to test for the significance of linear, quadratic and cubic components of the response to experimental treatments. Least-squares means are reported and treatment effects were declared significant at $P<0·05$.

Relationships between CaCLA in the diet, milk fatty acid composition, fat content and milk fat secretion were initially examined by regression analysis using the REG procedure of SAS. In cases where close linear or quadratic associations were identified between experimental variables, the relationship was further explored with an exponential decay model fitted using the Marquardt non-linear algorithm within the NLIN procedure of SAS[32].

Results

All animals remained in good health during the experiment, but in both experiments, goats did not consume all of the CaCLA supplement offered and therefore the amount of *trans*-10, *cis*-12 CLA supplied by experimental treatments was marginally lower than planned.

Experiment 1

Inclusion of CaCLA in the diet had no effect ($P>0·05$) on DM intake, milk yield, milk protein content, milk lactose concentrations or live weight, but increased linearly ($P<0·01$) fatty acid intake and decreased linearly ($P<0·01$) milk fat content and yield (Table 3). Compared with the control, treatments C1, C2 and C3 resulted in 19·8, 27·9 and 32·3 % decreases in milk fat yield and 16·2, 22·7 and 29·4 % reductions in milk fat content, respectively. Reductions in milk fat synthesis to CaCLA were also accompanied by changes in milk fatty acid composition characterised by linear ($P<0·05$) decreases in 6:0, 8:0, 10:0, 12:0, *cis*-18:1, 20:2*n*-6, 20:4*n*-6, 20:5*n*-3 and 22:5*n*-3 and linear ($P<0·05$) increases in 18:0, Σ *trans*-18:1, Σ CLA, 20:0, 22:0 and Σ PUFA concentrations (Table 4). CaCLA supplements also induced linear or quadratic ($P<0·05$) decreases in the concentration of fatty acids containing a *cis*-9 double bond, with the exception of *cis*-9, *trans*-13-18:2, and reduced product:substrate ratios for Δ9-desaturase (Table 4). Concentrations of CLA isomers in milk other than *trans*-11, *cis*-13 were enhanced linearly ($P<0·05$) in response to incremental inclusion of CaCLA in the diet (Table 5).

Incremental inclusion of CaCLA in the diet decreased linearly ($P<0·05$) fatty acid secretion in milk due to reductions in both the output of ≤C16 fatty acids synthesised *de novo* and C16 fatty acids (Fig. 1 (a)). CaCLA supplements had no effect ($P>0·05$) on the secretion of > C18 long-chain fatty acids in milk derived from the uptake of circulating plasma lipids (Fig. 1 (a)). Experimental treatments increased linearly ($P<0·001$) *trans*-10, *cis*-12 CLA output in milk from 14·7 to 163, 296 and 404 mg/d, associated with a mean efficiency of transfer from the diet into milk of 2·37, 2·38 and 2·19 % (SE 0·099, $P=0·412$) for treatments C1, C2 and C3, respectively.

Experiment 2

Substituting CaCLA for CaPO in the diet had no effect ($P>0·05$) on DM intake, milk yield, milk protein content, milk lactose concentrations or live weight, but decreased linearly ($P<0·01$) milk fat content, milk fat output and fat-corrected milk yield (Table 6). Relative to the control, replacing CaPO with CaCLA in the diet resulted in 17·5, 39·0 and 49·3 % decreases in milk fat yield and 24·0, 33·8 and 35·8 % reductions in milk fat content for treatments CP1, CP2 and CP3, respectively. Supplements CaPO and CaCLA were declared as containing the same amount of fatty acids. Due to measured differences in the supplement lipid content (Table 1), and marginal decreases in forage and concentrate DM intake, incremental replacement of CaPO with CaCLA in the diet resulted in a linear reduction ($P<0·05$) in total fatty acid ingestion (Table 6). Replacing CaPO with CaCLA in the diet also altered milk fatty acid composition characterised by linear ($P<0·05$) decreases in 6:0, 8:0, 10:0, 12:0, 16:0 and 20:4*n*-6 and linear or quadratic ($P<0·05$) increases in 18:0, Σ *trans*-18:1, Σ CLA, 18:3*n*-3, 20:0, 20:2*n*-6, 22:0 and Σ PUFA concentrations (Table 7). Substituting CaPO with CaCLA in the diet also induced linear or quadratic ($P<0·05$) decreases in the concentration of fatty acids containing a *cis*-9 double bond, other than *cis*-9, *trans*-13-18:2 and reduced product:substrate ratios for Δ9-desaturase (Table 7). Incremental inclusion of CaCLA at the expense of CaPO also enhanced in a linear or quadratic manner ($P<0·05$) the concentration of all CLA isomers in milk, with the exception of *trans*-11, *cis*-13 (Table 8).

Replacement of CaPO with CaCLA decreased linearly ($P<0·05$) milk fatty acid output, changes that were attributable to a decrease in ≤C14 and C16 fatty acids, while the secretion of > C18 long-chain fatty acids in milk was independent of experimental treatment (Fig. 1 (b)). Inclusion of

Table 3. Effect of dietary conjugated linoleic acid (CLA) supplements on nutrient intake, milk production and live weight in lactating goats (Experiment 1)*

	Treatment†					
	C0	C1	C2	C3	SEM‡	*P*§
Intake (g/d)						
Lucerne hay DM	1135	1203	1070	1220	91·5	0·775
Concentrate DM	640	615	530	497	18·1	<0·001
CaCLA	0·0	28·8	50·5	74·3	3·61	<0·001
Σ DM	1775	1825	1650	1793	75·8	0·694
12 : 0	0·11	0·11	0·10	0·11	0·008	0·860
14 : 0	0·18	0·23	0·24	0·30	0·010	<0·001
16 : 0	5·13	7·09	7·94	9·73	0·198	<0·001
cis-9-16 : 1	0·04	0·07	0·08	0·10	0·003	<0·001
18 : 0	0·70	1·41	1·87	2·48	0·078	<0·001
cis-9-18 : 1	4·25	6·74	8·24	10·2	0·430	<0·001
cis-11-18 : 1	0·15	0·34	0·47	0·62	0·026	<0·001
18 : 2*n*-6	9·18	9·01	7·92	7·77	0·157	<0·001
cis-9,*trans*-11 CLA	0·00	7·37	13·0	19·1	0·93	<0·001
trans-10,*cis*-12 CLA	0·00	6·87	12·2	17·8	0·87	<0·001
Σ CLA	0·00	15·9	28·2	41·2	2·01	<0·001
18 : 3*n*-3	2·52	2·63	2·34	2·59	0·155	0·895
20 : 0	0·14	0·14	0·12	0·13	0·006	0·236
22 : 0	0·12	0·19	0·23	0·29	0·006	<0·001
24 : 0	0·16	0·17	0·15	0·16	0·009	0·641
Other fatty acids	0·99	1·02	0·90	0·97	0·050	0·423
Σ Fatty acids	23·9	45·7	59·7	77·9	2·71	<0·001
Yield (g/d)						
Milk	1988	1900	1850	1913	67·7	0·399
Fat-corrected milk‖	2198	1900	1770	1740	72·3	0·004
Fat	82·9	66·5	59·8	56·1	3·28	0·001
Protein	76·8	73·3	69·5	71·7	2·67	0·167
Lactose	88·2	85·9	81·5	83·0	3·05	0·197
Concentration (g/kg)						
Fat	41·9	35·1	32·4	29·6	1·57	0·001
Protein	38·5	38·7	37·6	37·3	0·41	0·053
Lactose	44·5	45·1	44·1	43·4	0·63	0·184
Live weight (kg)	64·1	63·8	62·4	63·5	0·74	0·351

CaCLA, calcium salts of conjugated linoleic acid.
* Values represent the mean of days 9 and 10 of treatment.
† Diets based on lucerne hay supplemented with concentrates formulated to supply 0, 30, 60 or 90 g CaCLA/d (C0, C1, C2 and C3, respectively).
‡ Standard error of the mean for sixteen measurements; error df = 6.
§ Significance of linear responses to CaCLA supplements in the diet. Quadratic and cubic responses to CaCLA were NS ($P>0·05$).
‖ 35 g/kg fat-corrected milk yield (kg) = milk yield (kg) × (313 + 11·2 × fat content (g/kg)) / 704[51].

CaCLA in the diet increased linearly ($P<0·001$) *trans*-10, *cis*-12 CLA secretion in milk from 14·7 to 139, 278 and 219 mg/d for treatments C1, C2 and C3, responses associated with a mean apparent efficiency of transfer from the diet into milk of 2·22, 2·39 and 1·69 % (SE 0·381, $P=0·634$), respectively.

Discussion

Post-ruminal infusion studies have established a central role of *trans*-10, *cis*-12 CLA in the regulation of milk fat synthesis in the lactating cow[33,34]. Reductions in milk fat in response to abomasal infusions of *trans*-10, *cis*-12 CLA are known to occur in a predictable dose-dependent manner[32,33]. Further research has also demonstrated that post-ruminal infusions of a mixture of CLA isomers containing *trans*-9, *cis*-11[35] or *cis*-10, *trans*-12[36] as major components also exert anti-lipogenic effects in the bovine. Use of lipid-encapsulated supplements containing *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA has also provided evidence to suggest that the inhibitory effects of *trans*-10, *cis*-12 CLA are comparable in the lactating bovine and ovine[37,38]. By contrast, short-term administration of *trans*-10, *cis*-12 CLA in the peripheral circulation[10] or at the duodenum[11] was reported to have no effect on milk fat synthesis in the lactating goat, suggesting that the anti-lipogenic activity of *trans*-10, *cis*-12 CLA differs between ruminant species.

Numerous experiments in lactating cows have established that dietary supplements of calcium salts of a mixture of CLA isomers containing *trans*-10, *cis*-12 CLA inhibit milk fat synthesis during early or established lactation over a short or extended period[33]. Several studies have demonstrated that the anti-lipogenic potential of *trans*-10, *cis*-12 CLA supplied as CaCLA in the diet is lower immediately post-partum in the lactating cow[6,16,39]. Reduced inhibition of mammary lipogenesis does not appear to be related to variations in mammary supply and incorporation of *trans*-10, *cis*-12 CLA in milk fat[6,7,16] or specific changes in plasma glucose, insulin, leptin or NEFA concentrations[7,40]. It is possible that the coordinated reduction in the expression genes encoding for key lipogenic enzymes to *trans*-10, *cis*-12

Table 4. Effect of dietary conjugated linoleic acid (CLA) supplements on milk fatty acid composition in lactating goats (Experiment 1; g/100 g fatty acids)*

	Treatment†					*P*§	
	C0	C1	C2	C3	SEM‡	L	Q
4:0	1·64	1·86	1·91	1·83	0·053	0·039	0·031
6:0	2·00	1·98	1·80	1·72	0·041	0·002	0·542
8:0	2·28	2·16	1·84	1·73	0·067	<0·001	0·951
10:0	9·86	8·97	7·42	7·13	0·349	<0·001	0·419
cis-9-10:1	0·19	0·08	0·04	0·04	0·013	<0·001	0·005
12:0	6·12	5·16	4·18	4·06	0·194	<0·001	0·071
14:0	13·82	14·40	13·49	13·34	0·415	0·249	0·411
cis-9-14:1	0·27	0·13	0·08	0·05	0·010	<0·001	0·001
15:0	1·43	1·45	1·38	1·38	0·072	0·533	0·928
15:0 *iso*	0·23	0·25	0·24	0·24	0·010	0·800	0·313
15:0 *anteiso*	0·42	0·45	0·42	0·39	0·024	0·282	0·284
16:0	29·32	27·66	26·97	27·52	0·728	0·111	0·180
cis-9-16:1	0·83	0·49	0·43	0·37	0·028	<0·001	0·003
trans-9-16:1	0·07	0·13	0·21	0·27	0·031	0·003	0·986
trans-11-16:1	0·03	0·05	0·08	0·07	0·008	0·005	0·149
17:0	0·70	0·79	0·78	0·77	0·023	0·096	0·088
17:0 *iso*	0·37	0·44	0·50	0·54	0·028	0·003	0·521
cis-9-17:1	0·37	0·25	0·22	0·19	0·018	<0·001	0·035
18:0	6·32	9·68	12·02	11·74	0·438	<0·001	0·006
cis-9-18:1	15·65	11·89	11·32	9·96	0·647	<0·001	0·113
cis-11-18:1	0·33	0·36	0·39	0·42	0·021	0·027	0·909
cis-12-18:1	0·15	0·45	0·54	0·55	0·044	<0·001	0·018
cis-13-18:1	0·02	0·03	0·03	0·03	0·006	0·311	0·251
cis-15-18:1‖	0·14	0·16	0·16	0·17	0·006	0·037	0·681
trans-6,7 + 8-18:1	0·07	0·20	0·27	0·31	0·023	<0·001	0·128
trans-9-18:1	0·15	0·31	0·40	0·45	0·036	<0·001	0·175
trans-10-18:1	0·20	1·12	1·56	1·99	0·166	<0·001	0·191
trans-11-18:1	0·41	1·32	1·93	2·39	0·199	<0·001	0·316
trans-12-18:1	0·13	0·36	0·52	0·59	0·051	<0·001	0·160
trans-13 + 14 18:1	0·25	0·60	0·83	0·94	0·067	<0·001	0·123
trans-16-18:1¶	0·13	0·20	0·29	0·28	0·015	<0·001	0·023
Σ *Cis*-18:1	16·29	12·89	12·44	11·13	0·668	0·002	0·167
Σ *Trans*-18:1	1·34	4·11	5·80	6·95	0·538	<0·001	0·185
Σ 18:1	17·64	16·99	18·24	18·08	0·861	0·529	0·787
18:2*n*-6	1·83	1·75	1·82	1·75	0·057	0·511	0·911
cis-9, *trans*-12-18:2	0·05	0·12	0·19	0·22	0·028	0·003	0·540
cis-9, *trans*-13-18:2	0·13	0·14	0·14	0·10	0·014	0·167	0·184
Σ CLA	0·47	1·53	2·69	3·77	0·409	<0·001	0·979
18:3*n*-3	0·50	0·50	0·50	0·49	0·029	0·813	0·970
20:0	0·12	0·16	0·18	0·17	0·006	0·001	0·011
20:2*n*-6	0·004	0·014	0·016	0·028	0·0061	0·035	0·844
20:3*n*-6	0·012	0·008	0·007	0·002	0·0034	0·109	0·830
20:4*n*-6	0·14	0·10	0·10	0·09	0·004	<0·001	0·014
20:5*n*-3	0·08	0·06	0·05	0·05	0·005	0·004	0·113
22:0	0·04	0·06	0·06	0·06	0·005	0·042	0·154
22:5*n*-3	0·11	0·09	0·08	0·07	0·006	0·002	0·803
Summary							
≤C14	36·9	35·3	31·2	30·4	0·83	<0·001	0·687
C16	30·5	28·6	27·9	28·5	0·70	0·076	0·131
≥ C18	27·4	31·2	36·1	36·7	1·51	0·003	0·331
Σ Saturates	75·7	76·4	74·0	73·4	1·12	0·114	0·597
Σ MUFA	19·4	18·1	19·3	19·1	0·09	0·966	0·573
Σ PUFA	3·34	4·31	5·59	6·57	0·402	<0·001	0·981
Σ Fatty acids (g/100 g fat)**	92·8	93·2	93·3	93·6	0·131	0·006	0·583
Ratio							
cis-9-10:1:10:0	0·019	0·009	0·005	0·005	0·0012	<0·001	0·004
cis-9-14:1:14:0	0·019	0·009	0·006	0·004	0·0006	<0·001	<0·001
cis-9-16:1:16:0	0·028	0·018	0·016	0·014	0·0011	<0·001	0·009
cis-9-17:1:17:0	0·537	0·323	0·274	0·241	0·0223	<0·001	0·007
cis-9-18:1:18:0	2·547	1·231	0·956	0·860	0·1202	<0·001	0·002
cis-9,*trans*-11 CLA:*trans*-11-18:1	0·644	0·512	0·562	0·622	0·0275	0·898	0·013

CaCLA, calcium salts of conjugated linoleic acid.
* Milk fatty acid profile on day 10 of treatment.
† Diets based on lucerne hay supplemented with concentrates formulated to supply 0, 30, 60 or 90 g CaCLA/d (C0, C1, C2 and C3, respectively).
‡ Standard error of the mean for sixteen measurements; error df = 6.
§ Significance of linear (L) and quadratic (Q) responses to CaCLA supplements in the diet. Cubic responses to CaCLA were NS ($P > 0·05$).
‖ Containing *trans*-17-18:1 as a minor component.
¶ Coeluting with *cis*-14-18:1 as a minor isomer.
** Fatty acid content of milk fat calculated assuming that lipid in milk is secreted as TAG.

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Table 5. Effect of dietary conjugated linoleic acid supplements on milk conjugated linoleic acid isomer concentrations in lactating goats (Experiment 1; mg/100 g fatty acids)*

	Treatment†					
	C0	C1	C2	C3	SEM‡	*P*§
cis-8, *cis*-10	2·7	2·9	5·4	12·2	2·89	0·053
cis-9, *cis*-11	6·9	34·2	57·3	82·7	8·26	<0·001
cis-10, *cis*-12	2·1	27·9	49·4	68·8	6·70	<0·001
cis-9, *trans*-11	265	658	1073	1459	135·2	<0·001
cis-11, *trans*-13	56·8	16·6	29·7	44·0	30·04	0·856
trans-7, *cis*-9	20·1	26·7	33·6	36·5	3·09	0·007
trans-8, *cis*-10	13·2	52·2	94·9	139	13·76	<0·001
trans-9, *cis*-11	15·5	35·7	56·5	77·3	10·93	<0·001
trans-10, *cis*-12	18·4	260	530	810	90·3	<0·001
trans-11, *cis*-13	6·1	2·7	4·2	5·9	3·09	0·967
trans-12, *cis*-14	0·8	3·6	4·6	6·1	1·48	0·042
trans-13, *cis*-15	1·6	3·5	4·6	6·7	0·97	0·009
trans-6, *trans*-8	5·8	44·1	84·6	118	14·77	0·001
trans-7, *trans*-9	3·1	20·2	41·8	56·1	6·69	<0·001
trans-8, *trans*-10	2·3	19·4	28·8	40·0	3·74	<0·001
trans-9, *trans*-11	18·8	139	254	353	43·3	0·001
trans-10, *trans*-12	13·9	148	270	366	41·7	<0·001
trans-11, *trans*-13	7·7	24·7	49·5	68·4	9·11	0·002
trans-12, *trans*-14	9·1	9·7	15·1	19·2	3·08	0·041

CaCLA, calcium salts of conjugated linoleic acid.
* Milk fatty acid profile on day 10 of treatment.
† Diets based on lucerne hay supplemented with concentrates formulated to supply 0, 30, 60 or 90 g CaCLA/d (C0, C1, C2 and C3, respectively).
‡ Standard error of the mean for sixteen measurements; error df = 6.
§ Significance of linear responses to CaCLA supplements in the diet. Quadratic and cubic responses to CaCLA were NS ($P>0·05$).

CLA is prevented due to the attenuation of cellular signalling systems during the onset of lactation[6,16]. A lower sensitivity of mammary lipogenesis to *trans*-10, *cis*-12 CLA during early lactation in the bovine is analogous to the response to *trans*-10, *cis*-12 CLA infusion in the goat[10,11], suggesting some common features in the regulation of mammary lipid metabolism. However, there are also several distinct differences between species: (i) decreases in milk fat synthesis in the bovine during early lactation involve a reduction in fatty acids synthesised *de novo* and preformed fatty acids, whereas reductions in milk fat synthesis to CaCLA in the goat were confined to ≤C14 and C16 fatty acids (Fig. 1), (ii) comparisons between experiments indicated that the inhibitory effects of CaCLA on milk fat synthesis in the goat are dependent on the supply of long-chain fatty acids (Fig. 2), whereas limited data in the lactating cow suggest that responses to *trans*-10, *cis*-12 CLA are independent of dietary fatty acid content[41] and (iii) a lower sensitivity to *trans*-10, *cis*-12 CLA in the bovine also coincides with alterations in several key enzymes and biochemical pathways at the onset of lactation and is a transitory phenomenon, whereas the lower sensitivity in the goat occurs in established lactation during periods of positive energy and nitrogen balance (Fig. 3).

The present experiment provided further evidence that CaCLA supplements also decrease milk fat content and

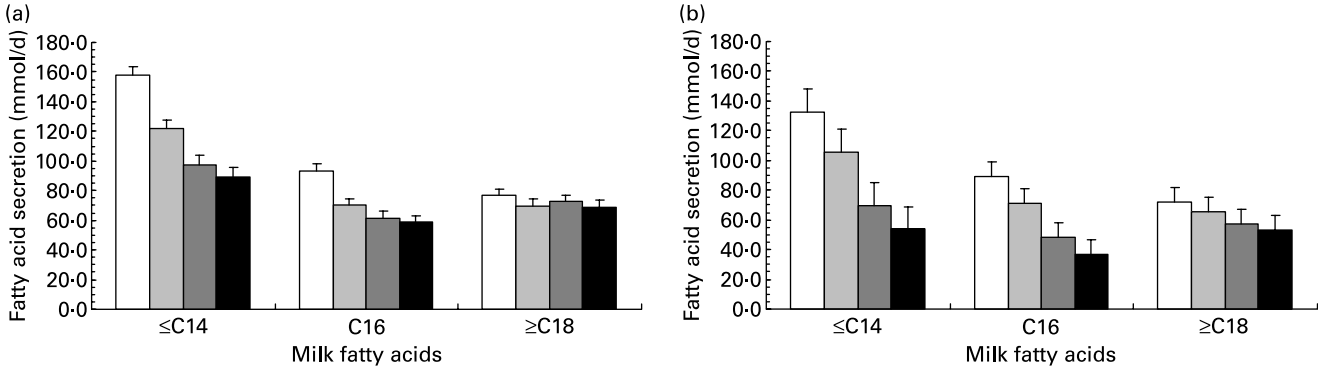

Fig. 1. Milk fatty acid secretion in goats measured on day 10 of treatment in response to (a) 0, 30, 60 or 90 g calcium salts of conjugated linoleic acid (CLA)/d in the diet (C0 (□), C1 (▨), C2 (▩) and C3 (■), respectively) or (b) incremental replacement of calcium salts of palm oil fatty acids in the diet with 0, 30, 60 or 90 g calcium salts of CLA/d (CP0 (□), CP1 (▨), CP2 (▩) and CP3 (■), respectively). Fatty acids in milk categorised according to metabolic origin: ≤C14 synthesised *de novo*; ≥ C18 extracted and incorporated into milk fat from circulating plasma lipids; C16 derived from both sources. For both experiments, dietary supplements of calcium salts of CLA decreased linearly ($P<0·01$) ≤C14 and ≥ C16 output but had no effect ($P>0·05$) on ≥ C18 secretion in milk. Quadratic and cubic responses to experimental treatments were NS ($P>0·05$). Error bars indicate standard errors of the mean for sixteen measurements.

Table 6. Effect of replacing calcium salts of palm oil fatty acids in the diet with supplements of conjugated linoleic acid (CLA) on nutrient intake, milk production and live weight in lactating goats (Experiment 2)*

	Treatment†					
	CP0	CP1	CP2	CP3	SEM‡	*P*§
Intake (g/d)						
Lucerne hay DM	1180	1250	1208	1018	121·2	0·368
Concentrate DM	510	518	473	433	32·9	0·108
CaPO	76·3	52·3	23·5	0·0	2·30	<0·001
CaCLA	0·0	26·0	46·3	63·0	4·89	<0·001
Σ DM	1765	1848	1748	1515	154·7	0·269
12 : 0	0·38	0·30	0·19	0·09	0·017	<0·001
14 : 0	1·25	0·96	0·58	0·25	0·049	<0·001
16 : 0	38·2	29·5	17·9	8·26	1·45	<0·001
cis-9-16 : 1	0·16	0·14	0·11	0·09	0·009	<0·001
18 : 0	3·09	2·98	2·52	2·11	0·206	0·010
cis-9-18 : 1	25·6	21·0	14·2	8·77	1·081	<0·001
cis-11-18 : 1	0·12	0·30	0·43	0·53	0·041	<0·001
18 : 2*n*-6	13·0	11·5	8·98	6·77	0·624	<0·001
cis-9,*trans*-11 CLA	0·00	6·68	11·9	16·1	1·255	<0·001
trans-10,*cis*-12 CLA	0·00	6·23	11·1	15·1	1·170	<0·001
Σ CLA	0·00	14·4	25·7	34·9	2·71	<0·001
18 : 3*n*-3	2·69	2·78	2·60	2·17	0·246	0·169
20 : 0	0·25	0·22	0·16	0·11	0·014	<0·001
22 : 0	0·12	0·18	0·22	0·25	0·021	0·004
24 : 0	0·16	0·17	0·16	0·14	0·015	0·307
Other fatty acids	1·65	1·47	1·16	0·82	0·099	<0·001
Σ Fatty acids	86·9	86·6	75·7	66·3	6·12	0·037
Yield (g/d)						
Milk	1650	1813	1488	1413	115·2	0·091
Fat-corrected milk‖	1905	1773	1375	1223	179·7	0·023
Fat	73·8	60·9	45·0	37·4	8·33	0·015
Protein	59·9	65·0	54·2	50·7	4·02	0·076
Lactose	71·1	77·7	63·3	61·2	5·27	0·111
Concentration (g/kg)						
Fat	44·1	33·5	29·2	28·3	2·61	0·005
Protein	36·6	36·6	37·1	36·3	0·52	0·832
Lactose	42·9	42·1	40·6	42·6	0·68	0·463
Live weight (kg)	61·1	60·4	58·9	59·4	0·74	0·091

CaCLA, calcium salts of CLA; CaPO, calcium salts of palm oil fatty acids.

* Values represent the mean of days 9 and 10 of treatment.

† Diets based on lucerne hay supplemented with concentrates containing calcium salts of palm oil fatty acids that were replaced incrementally with calcium salts of conjugated linoleic acid to supply 0, 30, 60 or 90 g CaCLA/d (CP0, CP1, CP2 and CP3, respectively).

‡ Standard error of the mean for sixteen measurements; error df = 6.

§ Significance of linear responses to CaCLA supplements in the diet. Quadratic and cubic responses to CaCLA were NS ($P>0·05$).

‖ 35 g/kg fat-corrected milk yield (kg) = milk yield (kg) × (313 + 11·2 × fat content (g/kg))/704[51].

yield in the lactating goat in a dose-dependent manner (Fig. 2). A specific reduction in milk fat secretion in the absence of changes in milk yield, milk protein content or lactose concentrations is consistent with typical milk production responses to CaCLA reported for lactating cows over short or extended periods[42]. However, the changes in milk fat synthesis were dependent on CaCLA supplementation regimen. Increases in *trans*-10, *cis*-12 CLA intake from CaCLA in Experiment 1 were associated with a curvilinear decrease in milk fat yield and content, whereas in Experiment 2, CaCLA treatments resulted in a linear decrease in milk fat yield and a curvilinear reduction in milk fat content (Fig. 2).

Relative reductions in milk fat secretion were larger when CaCLA replaced CaPO in the diet than when offered alone, which can be attributed to incremental replacement of CaPO with CaCLA resulting in marginal linear reductions in total fatty acid intake (Table 6), arising at least in part, from non-significant decreases in DM intake. Inclusion of CaCLA in the diet enhanced linearly dietary fatty acid intake, with the implication that the supply of 18:0 available for absorption was also increased due to extensive metabolism of the CaCLA supplement in the rumen as inferred from a low transfer of *trans*-10, *cis*-12 CLA into milk. In marked contrast to the bovine, increases in the supply of dietary fatty acids are known to enhance milk fat content in the goat[8,9], indicating that alterations in dietary fatty acid supply may also contribute to between-experiment variation in milk fat responses to CaCLA. Milk fat content was higher (2·2 g/kg) for C0 than for CP0 treatments. Accounting for measured differences in milk composition between animals assigned to experiments indicated that CaPO in the diet elicited a mean 4·3 g/kg increase in milk fat concentrations consistent with responses of 3·7–5·2 g/kg reported in the literature[8]. Due to the critical role of fatty acid supply in the regulation of mammary lipogenesis in the caprine, the changes in milk fat content and secretion to substituting CaPO for CaCLA in the diet can be

Table 7. Effect of replacing calcium salts of palm oil fatty acids in the diet with supplements of conjugated linoleic acid (CLA) on milk fatty acid composition in lactating goats (Experiment 2; g/100 g fatty acids)*

	Treatment†					*P*§		
	CP0	CP1	CP2	CP3	SEM‡	L	Q	C
4:0	1·61	2·13	2·04	2·01	0·092	0·036	0·024	0·154
6:0	2·24	2·10	1·82	1·73	0·148	0·034	0·863	0·641
8:0	2·56	2·14	1·78	1·65	0·191	0·011	0·488	0·835
10:0	10·08	8·33	6·98	6·19	0·820	0·012	0·581	0·963
cis-9-10:1	0·30	0·08	0·06	0·07	0·017	<0·001	<0·001	0·077
12:0	5·69	4·54	4·14	3·55	0·561	0·034	0·631	0·718
14:0	12·17	13·45	13·24	11·39	1·394	0·698	0·304	0·984
cis-9-14:1	0·33	0·11	0·10	0·10	0·022	<0·001	0·003	0·099
15:0	1·14	1·04	1·04	1·03	0·087	0·422	0·637	0·788
15:0 *iso*	0·16	0·17	0·17	0·18	0·009	0·262	0·985	0·866
15:0 *anteiso*	0·27	0·30	0·27	0·29	0·025	0·909	0·889	0·405
16:0	31·25	30·59	28·39	25·53	1·062	0·007	0·339	0·859
cis-9-16:1	1·00	0·46	0·46	0·51	0·044	<0·001	<0·001	0·051
trans-9-16:1	0·06	0·09	0·16	0·22	0·025	0·003	0·572	0·647
trans-11-16:1	0·02	0·05	0·06	0·08	0·008	0·001	0·533	0·486
17:0	0·45	0·62	0·70	0·85	0·102	0·032	0·918	0·757
17:0 *iso*	0·25	0·32	0·37	0·46	0·025	<0·001	0·635	0·692
cis-9-17:1	0·25	0·16	0·21	0·27	0·052	0·673	0·220	0·654
18:0	4·81	8·86	10·57	12·33	0·953	0·001	0·272	0·596
cis-9-18:1	17·47	14·17	14·22	16·62	3·050	0·861	0·386	0·944
cis-11-18:1	0·31	0·34	0·38	0·43	0·027	0·014	0·770	0·972
cis-12-18:1	0·13	0·35	0·51	0·56	0·063	0·002	0·260	0·872
cis-13-18:1	0·03	0·02	0·02	0·04	0·004	0·308	0·008	0·394
cis-15-18:1‖	0·11	0·12	0·13	0·15	0·009	0·017	0·550	0·689
trans-6,7 + 8-18:1	0·11	0·19	0·22	0·27	0·029	0·008	0·690	0·633
trans-9-18:1	0·19	0·30	0·37	0·46	0·020	<0·001	0·619	0·554
trans-10-18:1	0·22	0·78	1·21	1·42	0·161	0·001	0·300	0·897
trans-11-18:1	0·35	0·82	1·32	1·75	0·180	0·001	0·908	0·880
trans-12-18:1	0·13	0·30	0·40	0·51	0·046	0·001	0·528	0·750
trans-13 + 14 18:1	0·27	0·57	0·69	0·81	0·069	0·001	0·255	0·573
trans-16-18:1¶	0·12	0·17	0·22	0·28	0·013	<0·001	0·804	0·647
Σ *cis*-18:1	18·05	14·99	15·26	17·80	3·031	0·973	0·391	0·941
Σ *trans*-18:1	1·39	3·13	4·44	5·50	0·494	<0·001	0·512	0·938
Σ 18:1	19·44	18·13	19·70	23·30	2·657	0·311	0·390	0·944
18:2*n*-6	2·19	2·29	2·39	2·42	0·160	0·306	0·816	0·923
cis-9, *trans*-12-18:2	0·05	0·09	0·14	0·17	0·011	<0·001	0·951	0·596
cis-9, *trans*-13-18:2	0·12	0·11	0·12	0·14	0·011	0·188	0·146	0·804
Σ CLA	0·45	1·38	2·62	2·90	0·298	<0·001	0·322	0·380
18:3*n*-3	0·40	0·47	0·53	0·54	0·041	0·034	0·516	0·802
20:0	0·10	0·14	0·14	0·14	0·007	0·019	0·032	0·374
20:2*n*-6	0·008	0·017	0·022	0·024	0·0024	0·003	0·248	0·888
20:3*n*-6	0·010	0·007	<0·001	0·006	0·0032	0·213	0·216	0·283
20:4*n*-6	0·12	0·10	0·10	0·10	0·004	0·028	0·097	0·049
20:5*n*-3	0·06	0·05	0·05	0·05	0·005	0·165	0·319	0·575
22:0	0·02	0·03	0·03	0·03	0·002	0·017	0·078	0·285
22:5*n*-3	0·08	0·07	0·08	0·07	0·004	0·358	0·980	0·218
Summary								
≤C14	35·6	33·3	30·6	27·0	2·93	0·072	0·845	0·979
C16	32·5	31·4	29·3	26·5	1·03	0·005	0·472	0·928
≥ C18	27·9	31·7	36·5	42·3	3·48	0·022	0·794	0·995
Σ Saturates	73·7	75·4	72·3	68·0	2·61	0·135	0·289	0·770
Σ MUFA	21·4	19·1	20·7	24·5	2·69	0·394	0·297	0·883
Σ PUFA	3·49	4·56	6·03	6·42	0·215	<0·001	0·165	0·170
Σ Fatty acids (g/100 g fat)**	92·9	93·5	93·6	93·5	0·117	0·010	0·052	0·764
Ratio								
cis-9-10:1:10:0	0·030	0·009	0·008	0·012	0·0013	<0·001	<0·001	0·052
cis-9-14:1:14:0	0·027	0·008	0·007	0·009	0·0013	<0·001	<0·001	0·059
cis-9-16:1:16:0	0·032	0·015	0·016	0·021	0·0020	0·010	0·002	0·175
cis-9-17:1:17:0	0·568	0·262	0·291	0·298	0·0302	0·001	0·002	0·039
cis-9-18:1:18:0	3·807	1·622	1·414	1·313	0·2351	<0·001	0·004	0·126
cis-9, *trans*-11 CLA:*trans*-11-18:1	0·902	0·750	0·884	0·780	0·0355	0·194	0·517	0·016

CaCLA, calcium salts of conjugated linoleic acid.
* Milk fatty acid profile on day 10 of treatment.
† Diets based on lucerne hay supplemented with concentrates containing calcium salts of palm oil fatty acids that were replaced incrementally with calcium salts of conjugated linoleic acid to supply 0, 30, 60 or 90 g CaCLA/d (CP0, CP1, CP2 and CP3, respectively).
‡ Standard error of the mean for sixteen measurements; error df = 6.
§ Significance of linear (L), quadratic (Q) and cubic (C) responses to CaCLA supplements in the diet.
‖ Containing *trans*-17-18:1 as a minor component.
¶ Coeluting with *cis*-14-18:1 as a minor isomer.
** Fatty acid content of milk fat calculated assuming that lipid in milk is secreted as TAG.

Table 8. Effect of replacing calcium salts of palm oil fatty acids with supplements of conjugated linoleic acid on milk conjugated linoleic acid isomer concentrations in lactating goats (Experiment 2; mg/100 g fatty acids)*

	Treatment†					*P*§	
	CP0	CP1	CP2	CP3	SEM‡	L	Q
cis-8,*cis*-10	0·4	4·8	5·5	5·5	0·90	0·007	0·048
cis-9,*cis*-11	4·8	31·2	59·5	63·2	7·98	0·001	0·204
cis-10,*cis*-12	2·0	23·5	46·0	46·3	7·13	0·003	0·188
cis-9,*trans*-11	310	607	1142	1316	101·9	<0·001	0·568
cis-11,*trans*-13	1·9	15·2	32·7	37·5	3·69	<0·001	0·296
trans-7,*cis*-9	31·9	26·7	32·8	43·8	3·26	0·028	0·049
trans-8,*cis*-10	11·1	47·6	103	109	11·27	<0·001	0·229
trans-9,*cis*-11	13·3	26·8	45·8	68·6	5·44	<0·001	0·429
trans-10,*cis*-12	20·6	248	571	580	87·8	0·002	0·259
trans-11,*cis*-13	1·4	2·2	4·2	3·3	0·83	0·086	0·345
trans-12,*cis*-14	0·1	1·8	3·9	5·3	0·80	0·003	0·918
trans-13,*cis*-15	1·0	2·3	3·3	6·1	0·71	0·002	0·325
trans-6,*trans*-8	4·8	38·3	66·2	76·3	6·91	<0·001	0·139
trans-7,*trans*-9	2·3	16·6	31·8	36·8	4·82	0·002	0·369
trans-8,*trans*-10	5·1	16·6	23·4	25·8	3·28	0·003	0·211
trans-9,*trans*-11	16·3	115	182	199	28·6	0·003	0·203
trans-10,*trans*-12	10·9	125	214	227	33·0	0·003	0·177
trans-11,*trans*-13	3·2	20·9	38·4	40·8	6·36	0·004	0·275
trans-12,*trans*-14	3·5	7·7	10·9	13·4	1·90	0·008	0·679

CaCLA, calcium salts of conjugated linoleic acid.
* Milk fatty acid profile on day 10 of treatment.
† Diets based on lucerne hay supplemented with concentrates containing calcium salts of palm oil fatty acids that were replaced incrementally with calcium salts of conjugated linoleic acid to supply 0, 30, 60 or 90 g CaCLA/d (CP0, CP1, CP2 and CP3, respectively).
‡ Standard error of the mean for sixteen measurements; error df = 6.
§ Significance of linear (L) and quadratic (Q) responses to CaCLA supplements in the diet. Cubic responses to CaCLA were NS ($P > 0·05$).

considered to be a close reflection of the anti-lipogenic potential of CaCLA supplements in the lactating goat.

CaCLA supplements contained several CLA isomers including *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA as major components. Due to a lack of experimental data in the goat, inferences on the possible contribution of constituent isomers to the observed reductions in milk fat have been drawn based on evidence from studies in the lactating cow. Post-ruminal infusion experiments have established that *cis*-9, *trans*-11 CLA, *cis*-11, *trans*-13 CLA, *trans*-8, *cis*-10 CLA, *trans*-9, *trans*-11 CLA and *trans*-10, *trans*-12 CLA are not involved in the regulation of milk fat synthesis in the bovine[33]. Inclusion of CaCLA in the diet was also associated with linear increases in milk fat *trans*-18:1 (Δ6–16) and *cis*-18:1 (Δ11, 12 and 15) concentrations. Administration of *trans*-9, -10, -11 and -12-18:1 and *cis*-11 and -12-18:1 at the duodenum have been shown to have no effect on milk fat synthesis in the lactating cows[33], while recent evaluations have in some[43], but not all, cases[25] suggested *trans*-7-18:1 as a putative milk fat inhibitor. Overall, a detailed analysis of the lipid composition of the CaCLA supplement coupled with the evidence from studies in lactating cows provides support for *trans*-10, *cis*-12 CLA being responsible for the reductions in milk fat synthesis in the goat determined in the present study, consistent with the

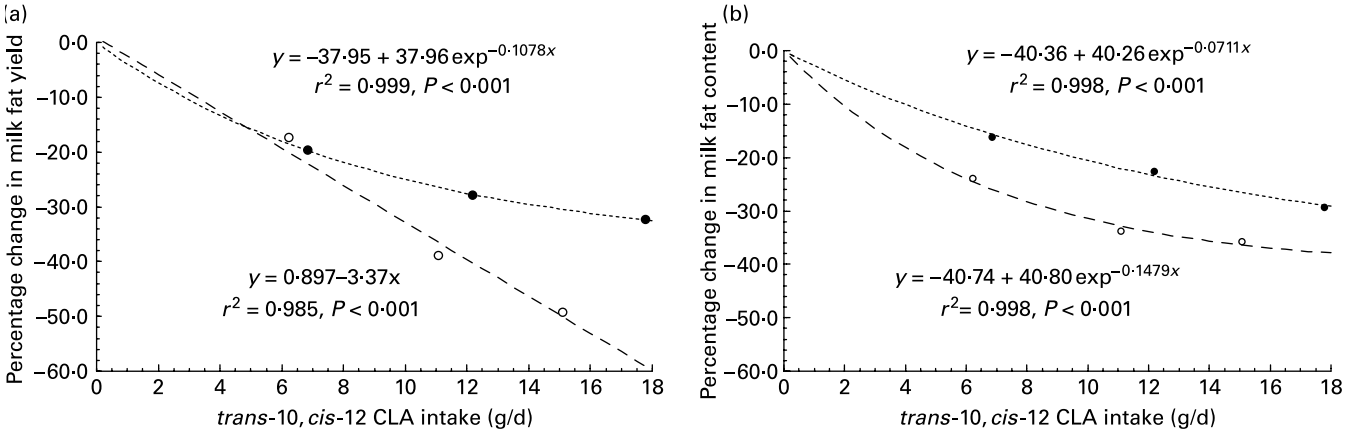

Fig. 2. Relationship between the percentage reduction in (a) milk fat yield and (b) milk fat content in lactating goats relative to a control diet in response to *trans*-10, *cis*-12 CLA intake. Treatments comprised calcium salts of CLA (●; experiment 1) or incremental replacement of calcium salts of palm oil fatty acids in the diet with calcium salts of CLA (○; experiment 2). For both experiments, symbols represent least-squares means (n 4).

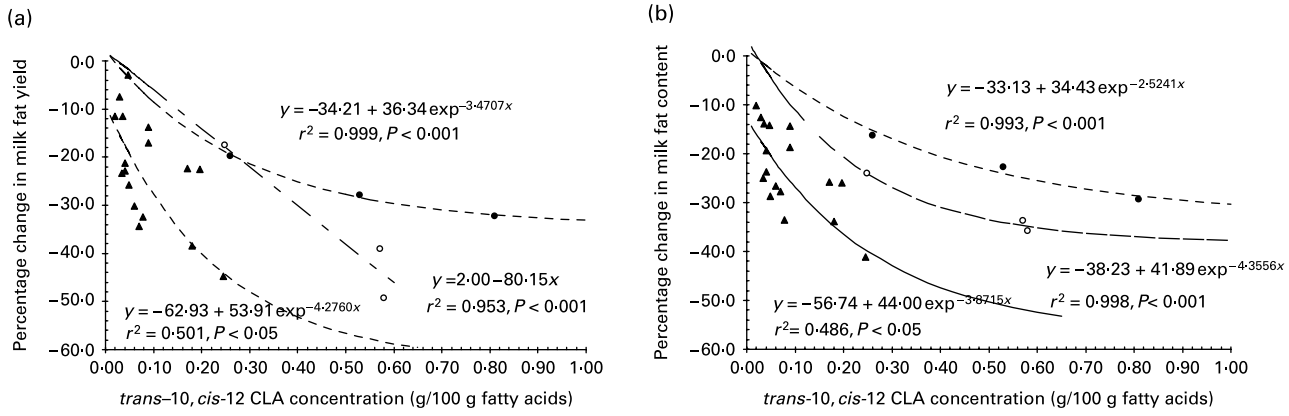

Fig. 3. Relationship between the percentage reduction in (a) milk fat yield and (b) milk fat content with milk fat *trans*-10,*cis*-12 CLA concentration in lactating goats relative to responses determined in lactating cows. Treatments comprised calcium salts of CLA (●; experiment 1) or incremental replacement of calcium salts of palm oil fatty acids in the diet with calcium salts of CLA (○; experiment 2). Symbols for experiments 1 and 2 represent least-squares means (*n* 4). Data in lactating cows (▲) derived from (*n* 11) studies reported in the literature[5–7,15–18,52–55] evaluating responses to calcium salts of CLA during early or established lactation.

well-established anti-lipogenic activity of this CLA isomer determined in other mammalian species[1,34].

Experimental CLA treatments also resulted in a dose-dependent increase in milk fat *trans*-9, *cis*-11 CLA concentrations. Supplements were devoid of *trans*-9, *cis*-11 CLA, indicating that this isomer was derived from metabolism of CaCLA during transit through the gastrointestinal tract, absorption or in recipient tissues. Previous studies in lactating cows have also demonstrated that CaCLA supplements enrich *trans*-9, *cis*-11 CLA concentrations in milk fat[33]. Calcium salts of fatty acids have been developed to minimise the impact of supplemental lipid on ruminal digestion and microbial protein synthesis but do not afford complete protection from biohydrogenation in the rumen[44,45]. *Trans*-9, *cis*-11 CLA is a transitory intermediate of 18 : 2*n*-6 metabolism in the rumen[33,46]. However, inclusion of CaCLA in the diet for both experiments decreased 18 : 2*n*-6 intake, suggesting that *trans*-9, *cis*-11 CLA incorporated into milk originated from metabolism of fatty acids in the CaCLA supplement. Studies in the lactating cow have shown that *trans*-9, *cis*-11 CLA inhibits milk fat synthesis with an estimated efficacy of 50 % compared with *trans*-10, *cis*-12 CLA[35]. While significant, the small increases in milk fat *trans*-9, *cis*-11 CLA content to CaCLA treatments would tend to suggest a relatively minor contribution to the observed reductions in milk fat synthesis in the present study in goats.

The apparent discrepancy in milk fat responses to CaCLA determined in the present experiment compared with a lack of effect to intravenous[10] or duodenal[11] infusions of *trans*-10, *cis*-12 CLA reported in earlier studies in goats may have several causes. Both intravenous and abomasal infusions were established over a short interval (2 and 3 d, respectively), while the incorporation of *trans*-10, *cis*-12 CLA in milk fat was much lower compared with the present data. It is possible that both the duration and amount of *trans*-10, *cis*-12 CLA infused were insufficient for the expression of anti-lipogenic activity in the caprine mammary gland. However, 72 h abomasal infusions have been shown to exert significant anti-lipogenic potential in the lactating bovine[2,3], suggesting that differences in the amount of *trans*-10, *cis*-12 CLA infused or from the diet in rumen-protected form are the more probable explanation. Previous studies examining the effect of a lipid-encapsulated supplement of CLA containing *trans*-10, *cis*-12 CLA in lactating goats provided tentative evidence indicating that the amount of supplement required to inhibit milk fat synthesis is higher compared with the lactating cow when species comparisons are made on the basis of live weight[12,13].

Irrespective of dietary fatty acid content, increases in *trans*-10, *cis*-12 CLA intake from CaCLA supplements resulted in curvilinear decreases in milk fat content (Fig. 2). Previous studies in lactating cows have established that the reduction in milk fat secretion to CaCLA[5–7] or post-ruminal infusions of relative pure preparations of *trans*-10, *cis*-12 CLA[32,33] also occurs in a dose-dependent non-linear manner. Such observations suggest that *trans*-10, *cis*-12 CLA acts via common mechanisms in ruminant species. The molecular mechanisms involved in the regulation of milk fat synthesis are not well defined, but studies in lactating cows have provided evidence that *trans*-10, *cis*-12 CLA decreases mammary tissue abundance of mRNA for lipogenic genes encoding for key enzymes involved in milk fat synthesis[33,34,47]. Due to a coordinated reduction in the expression of genes that encode for enzymes involved in *de novo* fatty acid synthesis, fatty acid uptake and transport, and triacylglycerol synthesis, it has been suggested that these changes are mediated via a pathway-specific central regulation of lipogenic gene expression with the SRBEP-1 transcription factor being identified as a possible candidate[34]. Studies in lactating cows[5–7,42] and sheep[37,38] have established that rumen-protected sources of *trans*-10, *cis*-12 CLA result in a reduction in the secretion of fatty acids derived from both *de novo* synthesis and circulating plasma lipids. By contrast, changes in milk fat composition in the lactating goat to CaCLA supplements determined in the present experiment revealed that the decrease in milk fat secretion was due to a reduction in fatty acids synthesised *de novo*, while the output of long-chain fatty acids was maintained, irrespective of dietary fatty acid intake. Differences in milk fatty acid composition point towards a lower inhibition of the uptake and incorporation of preformed fatty acids in response to *trans*-10, *cis*-12 CLA in the goat compared with the cow or sheep.

The mean apparent efficiency of transfer of *trans*-10, *cis*-12 CLA from CaCLA supplements into caprine milk averaged 2·03 %, which is within the range of values (1·9–7·4 %) reported for studies in lactating cows[15]. A lack of difference between experiments suggests that the incorporation of *trans*-10, *cis*-12 CLA into caprine milk is independent of the supply of essentially saturated fatty acids at the mammary gland. Furthermore, comparable efficiencies of transfer determined in the goat and cow would tend to suggest that the digestion, absorption and partitioning of *trans*-10, *cis*-12 CLA supplied in rumen-protected form as calcium salts is comparable among ruminant species. Indirect comparisons of the relationship between reductions in milk fat content and yield with milk *trans*-10, *cis*-12 CLA enrichment (Fig. 3) determined in the present study for the goat with data from studies in lactating cows fed diets containing CaCLA supplements point towards species differences being related to reduced sensitivity of mammary lipogenesis to *trans*-10, *cis*-12 CLA rather than metabolism of CaCLA in the rumen. Post-ruminal infusion studies in lactating cows have established that *trans*-10, *cis*-12 CLA induces a maximal inhibition of milk fat synthesis of approximately 50 %[32–34]. Assuming that the incorporation in milk fat reflects the supply at the mammary gland, comparisons of milk *trans*-10, *cis*-12 CLA concentrations between ruminants fed CaCLA supplements causing a predicted 25 % reduction in milk fat secretion (Fig. 3) suggest that the goat is between 4·1- and 4·8-fold less sensitive to the anti-lipogenic effects relative to the cow. Additional *in vitro* and *in vivo* studies examining the role of *trans*-10, *cis*-12 CLA on mRNA abundance on key lipogenic enzymes in caprine and bovine mammary tissue are required to identify the underlying causative mechanisms for these differences.

CaCLA supplements also induced decreases in the concentration ratios of product:substrate for Δ9-desaturase in milk fat. Milk fat *cis*-9-12:1:12:0, *cis*-9-14:1:14:0, *cis*-9-16:1:16:0 and *cis*-9-18:1:18:0 concentration ratios are known to be highly correlated with mRNA abundance and activity of Δ9-desaturase in the mammary gland of goats[47,48]. Previous studies have established that *trans*-10, *cis*-12 CLA decreases mRNA abundance for Δ9-desaturase in bovine mammary tissue[49,50]. Measurements of milk fatty acid composition responses to CaCLA supplements also suggest that *trans*-10, *cis*-12 CLA down-regulates transcripts encoding for Δ9-desaturase in the caprine mammary gland. Earlier studies in the lactating goat reported that duodenal infusions of *trans*-10, *cis*-12 CLA decreased milk fat desaturase indices in the absence of an effect on milk fat synthesis[11], consistent with the view that inhibition of Δ9-desaturase activity is not, in isolation, sufficient to induce a reduction in milk fat synthesis[33].

In conclusion, CaCLA supplements containing *trans*-10, *cis*-12 CLA reduced milk fat synthesis in lactating goats in a dose-dependent manner. Reductions in milk fatty acid output were due to reductions in the secretion of fatty acids synthesised *de novo* rather than fatty acids derived from circulating plasma lipids, leading to a shift in milk fat composition containing higher proportions of long-chain fatty acids. Differences in the extent of inhibition on milk fat content and yield between experiments indicated that anti-lipogenic activity in the goat is dependent on both the supply of *trans*-10, *cis*-12 CLA and long-chain fatty acids available to the mammary gland. Indirect comparisons of the relationship between reductions in milk fat secretion and milk fat *trans*-10, *cis*-12 CLA content in response to CaCLA supplements point towards the sensitivity of mammary lipogenesis to the inhibitory effects of *trans*-10, *cis*-12 CLA being several-fold lower in the goat compared with the cow.

Acknowledgements

The authors gratefully acknowledge and appreciate the staff of the 'Les Cèdres' Animal Nutrition and Metabolism Unit, André Combeau, Christophe Mathevon and Denis Roux, in particular, for the diligent care of experimental animals and the technical assistance of Pierre Capitan, Didier Bany, Cyril Labonne, Piia Kairenius and Vesa Toivonen. All authors have contributed to the preparation of the paper and agree with the submitted manuscript content. There are no conflicts of interest.

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