Changes in Milk Fat in Response to Dietary Supplementation with Calcium Salts of *Trans*-18:1 or Conjugated Linoleic Fatty Acids in Lactating Dairy Cows

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ABSTRACT

Milk fat was investigated in lactating dairy cows fed diets supplemented with Ca salts of *trans* fatty acids (Ca-tFA) or Ca salts of conjugated linoleic acids (Ca-CLA). Forty-five Holstein cows (115 days in milk) were fed a control diet (51% forage; dry matter basis) supplemented with 400 g of EnerG II (Ca salts of palm oil fatty acids) for 2 wk; subsequently, 5 groups of 9 cows each were assigned for 4 wk to the control diet or diets containing 100 g of Ca-CLA or 100, 200, or 400 g of Ca-tFA in a randomized block design. Treatments had no effect on dry matter intake, milk production, protein, lactose, or somatic cell count. Milk fat percentage was reduced from 3.39% in controls to 3.30, 3.04, and 2.98%, respectively, by the Ca-tFA diets and to 2.54% by the Ca-CLA diet. Milk fat yield (1.24 kg/d in controls) was decreased by 60, 130, and 190 g/d with increasing dose of Ca-tFA and by 290 g/d with the Ca-CLA supplement. Consistent with increased endogenous synthesis of *cis*-9-containing CLA from precursors provided by the Ca-tFA diets, total CLA were similar in milk of cows fed Ca-CLA or Ca-tFA. Compared with controls, the Ca-CLA diet increased *trans*-10, *cis*-12-18:2 yield in milk, without altering levels of *trans*-18:1 isomers. In contrast, yields of most *trans*-18:1 isomers were elevated in milk of cows fed Ca-tFA diets, whereas yields of *trans*-10, *cis*-12-18:2 remained similar to control values. We conclude that milk fat depression can occur without an increase in *trans*-10, *cis*-12-18:2 in milk and that other components, perhaps the *trans*-10-18:1 isomer, may be involved.

(Key words: lactating cow, *trans* fatty acids, conjugated linoleic acids)

Abbreviation key: Ca-CLA = Ca salt of conjugated linoleic acids, Ca-tFA = Ca salt of *trans*-18:1 fatty acids, CLA = conjugated linoleic acids, FAME = fatty acid methyl ester, MFD = milk fat depression, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids, tFA = *trans*-18:1 fatty acids.

INTRODUCTION

Trans-18:1 fatty acids (tFA) and conjugated linoleic acids (CLA) produced during biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen are mixtures of positional isomers (Katz and Keeney, 1966; Griinari and Bauman, 1999), which subsequently are incorporated into milk fat of lactating cows (Parodi, 1977; Griinari and Bauman, 1999; Looor and Herbein, 2001; Piperova et al., 2002; Precht et al., 2002). Studies have demonstrated that levels of tFA (Kalscheur et al., 1997a; Griinari et al., 1998; Piperova et al., 2000) and CLA isomers (Piperova et al., 2000, 2002; Peterson et al., 2003) increase in milk fat of lactating cows fed a variety of diets associated with milk fat depression (MFD). Because of the active biohydrogenation process in the rumen, abomasal infusion has been the best approach to study effects of specific isomers on milk fat synthesis. Using abomasal infusion of *trans*-10, *cis*-12-18:2, Baumgard et al. (2000) provided evidence that this is one isomer involved in MFD. The role of tFA in MFD has been examined using partially hydrogenated vegetable oils, and we have previously shown (Gaynor et al., 1994; Romo et al., 1996) that abomasal infusion of partially hydrogenated vegetable oils in lactating cows results in elevated amounts of tFA in milk and a 14 to 25% reduction in milk fat percentage.

Griinari et al. (1998) and Griinari and Bauman (1999) reported a specific association between dietary-induced MFD and increased *trans*-10-18:1 in milk of lactating cows and found a linear relationship between *trans*-10-

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18:1 and *trans*-10, *cis*-12-18:2. Griinari and Bauman (1999) suggested that the *trans*-10-18:1 resulted from *trans*-10, *cis*-12-18:2 during rumen biohydrogenation, and recently Looor and Herbein (2001) provided direct evidence for this hypothesis. Subsequently, others have reported an increase in the concentrations of *trans*-10, *cis*-12-18:2 (Piperova et al., 2000; Peterson et al., 2003) and *trans*-10-18:1 (Newbold et al., 1998; Piperova et al., 2000; Piperova et al., 2002; Peterson et al., 2003) in milk of lactating cows fed MFD diets. Because of the concomitant increase in *trans*-10, *cis*-12-18:2 with *trans*-18:1 isomers, and especially *trans*-10-18:1, it has been difficult to demonstrate an independent effect of tFA during dietary-induced MFD.

Feeding diets supplemented with Ca salts of fatty acids is a practical way to bypass rumen biohydrogenation. Studies have already shown that diets supplemented with Ca salts of CLA (**Ca-CLA**) effectively reduce milk fat percentage and yield in lactating cows (Giesy et al., 2002; Perfield II et al., 2002; Bernal-Santos et al., 2003). Dietary Ca salts of tFA (**Ca-tFA**) supplementation could potentially increase the postprandial availability of *trans*-18:1 fatty acids and provide an alternative to abomasal infusion to test the potential effects of *trans*-18:1 fatty acids on milk fat synthesis. Hence, this study was undertaken to examine the effects of a dietary Ca-tFA or Ca-CLA supplementation on milk fat content and fatty acid isomer profile in lactating dairy cows.

MATERIALS AND METHODS

Animals, Treatments, and Sampling

The experiment was conducted according to a protocol approved by the University of Maryland Institutional Animal Care and Use Committee. The Ca-fatty acid supplements used in this study were provided by Bio-products Inc., (Fairlawn, OH). Forty-five lactating Holstein dairy cows (15 primiparous and 30 multiparous) at approximately 115 DIM were fed a control diet containing 51% forage and 49% concentrate (DM basis), supplemented with 400 g of EnerG II (Ca salts of palm oil fatty acids) during a 2-wk adjustment period. After the adjustment period, cows ($n = 9$ per group) were assigned to 1 of 5 treatments: control or 4 diets supplemented with either 100 g of Ca-CLA or with 100, 200, or 400 g of Ca-tFA in a randomized block design. In the treatment diets, the Ca-CLA and Ca-tFA supplements substituted for equal parts of EnerG II in the control diet. The amount of Ca-CLA fed in this study was based on results obtained by others using dietary Ca-CLA supplementation to reduce milk fat in lactating cows (Giesy et al., 2002; Perfield II et al., 2002). The 100 g of Ca-CLA supplement provided 13 g/d of total CLA,

Table 1. Fatty acid composition of Ca supplements.¹

Fatty acid	EnerG II	Ca-tFA	Ca-CLA
	g/100 g of fatty acid		
14:0			0.61
16:0	45.7	12.5	29.2
16:1			0.2
18:0	4.9	22.1	3.5
18:1 <i>trans</i>		53.3	
18:1 <i>cis</i>	38.5	8.3	29.5
18:2 n-6	9.8	1.8	17.6
18:2i ²		1.6	
CLA			18.5
Other	1.1	0.3	0.9

¹Control cows received a dietary supplement of Ca salts of palm oil fatty acids (EnerG II), providing 60 g/d of fatty acids/100 g of supplement. The Ca-tFA (Ca salt of *trans* fatty acids) and Ca-CLA (Ca salt of conjugated linoleic acids) treatments provided 60 and 70 g/d of fatty acids/100 g of supplement, respectively.

²18:2i = nonconjugated dienes with 1 or 2 *trans* double bonds.

containing 4.65 g of *trans*-10, *cis*-12-18:2 isomer, which is within the range used by others to achieve MFD. The Ca-tFA supplement provided 32, 64, and 128 g/d of tFA with the 100, 200, and 400 g of Ca-tFA, respectively. We used data from previous experiments, especially Kalscheur et al. (1997a) and Piperova et al. (2002), to estimate the expected increase in the amounts of tFA (~30 g/d) and *trans*-10-18:1 (~10 g/d) in milk fat of lactating cows exhibiting MFD. Assuming ~50% biohydrogenation (Wu et al., 1991; Wu and Palmquist, 1991) of the Ca-tFA in the rumen, we calculated that the 200-g dose of Ca-tFA should provide the levels required. The fatty acid compositions of the supplements are shown in Table 1, and the distribution pattern of the tFA and CLA isomers in the Ca supplements is given in Table 2.

The ingredient and chemical composition of the basal diet is shown in Table 3. Forage and concentrate DM was measured weekly, and the TMR was adjusted accordingly to maintain a constant forage-to-concentrate ratio on a DM basis during the experiment. Cows were housed in a free-stall barn and were fed individually.

Table 2. Isomer profile of the Ca-tFA (Ca salt of *trans* fatty acids) and Ca-CLA (Ca salt of conjugated linoleic acids) supplements.

<i>trans</i> -18:1 isomers (% of total tFA ¹)		CLA ² isomers (% of total CLA)	
<i>trans</i> -6-8	10.20	<i>trans</i> -11, <i>cis</i> -13	0.1
<i>trans</i> -9	16.20	<i>cis</i> -11, <i>trans</i> -13	11.50
<i>trans</i> -10	28.01	<i>trans</i> -10, <i>cis</i> -12	35.83
<i>trans</i> -11	20.01	<i>cis</i> -9, <i>trans</i> -11	35.08
<i>trans</i> -12	11.50	<i>trans</i> -8, <i>cis</i> -10	8.46
<i>trans</i> -13+14	10.50	<i>trans/trans</i> CLA	4.73
<i>trans</i> -15	1.82	<i>cis/cis</i> CLA	4.30
<i>trans</i> -16	0.92		

¹tFA = *Trans*-18:1 fatty acids.

²CLA = Conjugated linoleic acids.

Table 3. Ingredients and chemical composition of basal diet.

Ingredient	
	(% of DM)
Corn grain, ground	1.7
HMSC ¹	20.0
Soybean meal	14.3
Soybean roasted	3.8
Dried citrus pulp	5.3
Corn silage	42.5
Alfalfa hay	9.2
Dicalcium phosphate	0.5
KCl	0.5
NaCl	0.4
MgO	0.2
Potassium-magnesium sulfate	0.6
Trace minerals and vitamins ²	1.4
EnerG II ³	1.8
Estimated chemical composition	
DM, %	48.6
CP, %	17.6
RUP, %	6.8
ADF, %	17.7
NDF, %	30.2
Net energy for lactation, Mcal/kg	1.82
Ca, %	0.75
P, %	0.37
Na, %	0.31
Mg, %	0.30
S, %	0.15
K, %	1.32
Cl, %	0.41

¹HMSC = High moisture shelled corn.

²Combined 549 g/kg of calcium carbonate and 451 g/kg of trace mineral and vitamin mix. Trace mineral and vitamin mix combined (per kg mix) 24 g Mn, 24 g Zn, 6.5 g Cu, 0.29 g I, 0.048 g Co, 0.144 g Se, 0.95 g retinyl acetate, 0.018 g cholecalciferol, and 12.67 g DL-tocopheryl acetate.

³Ca salts of palm oil fatty acids (Bioproducts Inc., Fairlawn, OH).

Diets were fed as TMR once daily at 0800 h, refusals were measured, and DMI was calculated daily. Cows were milked, and milk production was recorded at 0600 and 1600 h every day. Milk samples were collected during the last 3 d (6 consecutive milkings) of the adjustment and the treatment periods and composited. Milk fat, protein, lactose, and somatic cell count were analyzed by infrared analyses (AOAC, 1990; Foss Milkoscan; Foss Food Technology Corp., Eden Prairie, MN).

Fatty Acid Composition

Milk fat was extracted, and fatty acid methyl esters (FAME) were prepared by a mild transesterification with 0.4 mol/L H₂SO₄ in methanol, as previously described (Pipero et al., 2002). The composition of FAME was determined by GLC analysis using a 60 m × 0.25 mm CP-Sil 88 fused-silica capillary column (Chrompack, Middelburg, The Netherlands) in a HP5890 II GLC system. Total tFA methyl ester was obtained after separation of FAME on Ag⁺-TLC (Sam-pugna et al., 1982), using FAME of 14:0, *cis*-9-18:1 and

trans-9-18:1 standards to locate the fractions of interest. *Trans* fatty acid isomers were separated on a 100 m × 0.25-mm fused-silica capillary column (SP-2560, Supelco Inc., Bellefonte, PA) in a GLC system and under conditions previously described (Pipero et al., 2000). Individual 18:1 isomers were identified with the help of authentic *cis*- and *trans*-18:1 fatty acids, as described by Pipero et al. (2000).

Milk FAME were analyzed for CLA content and isomer distribution using a combination of GLC and Ag⁺ HPLC as previously described (Pipero et al., 2000). Aliquots (60 µL) of each sample were diluted in 940 µL hexane and were used in HPLC analysis; a typical injection volume was 50 µL, containing 100 to 112 µg of FAME. Details on the identification and quantification of the CLA isomers by HPLC analysis have been reported elsewhere (Eulitz et al., 1999; Sehat et al., 1998).

Statistical Analysis

Data were analyzed using the Mixed Procedure of SAS (1998). The model included treatment as fixed effect and observations at the end of the adjustment period as covariate. The random portion of the model included the block effect. Tests of significance included linear and quadratic contrasts to test the effect of Ca-tFA dose (0, 100, 200, and 400 g), and contrasts for CLA vs. control and CLA vs. tFA treatments. Probability of *P* ≤ 0.05 was considered to be statistically significant.

RESULTS

Gas-liquid chromatography analysis of the Ca-tFA FAME revealed that *trans*-18:1 positional isomers with a double bond from delta-6 to delta-16 were present in the supplement, with *trans*-10 being the major isomer (Table 2). However, except for the *trans*-15 and *trans*-16-18:1, which were minor components, a considerable proportion of *trans*-11-18:1 was also present (20%), as well as from 10.2 to 11.5% of each of the remaining *trans*-18:1 components. In contrast, there was no evidence for *trans*-monoene in the Ca-CLA supplement. The Ca-CLA contained nearly equal amounts of *cis*-9, *trans*-11-18:2 (4.56 g) and *trans*-10, *cis*-12-18:2 (4.65 g), as well as several other isomers. However, there was no evidence for *trans*-7, *cis*-9-18:2 (Table 2). The Ca-tFA supplement was also examined for CLA, but only trace amounts of uncharacterized trienes and *trans*, *trans* dienes were detected (data not shown).

Dietary DMI, milk production, and milk composition data are presented in Table 4. Feed intake and milk yield were unaffected by dietary treatments. Milk protein and lactose content and yield were similar among

Table 4. Dry matter intake, milk production, and milk composition of cows fed diets supplemented with Ca salts of *trans* fatty acids (tFA) or conjugated linoleic acids (CLA).¹

Item	Control	Ca-tFA			Ca-CLA 100 g	SEM	L ²	Q ²	Contrast	
		100 g	200 g	400 g					CLA vs. control	CLA vs. tFA
DMI, kg/d	23.5	25.1	23.9	23.0	23.5	0.59	0.17	0.09	0.92	0.45
Milk yield, kg/d	37.8	35.9	36.2	36.4	35.2	1.36	0.67	0.61	0.33	0.24
Milk fat, %	3.39	3.30	3.04	2.98	2.54	0.06	0.03	0.76	0.01	0.01
Fat yield, kg/d	1.24	1.18	1.11	1.05	0.95	0.03	0.02	0.81	0.01	0.01
Milk protein, %	3.05	2.89	2.97	3.08	3.03	0.02	0.23	0.08	0.77	0.55
Protein yield, kg/d	1.09	1.04	1.08	1.08	1.14	0.19	0.90	0.69	0.37	0.13
Milk lactose, %	4.88	4.84	4.86	4.92	4.83	0.03	0.28	0.31	0.46	0.35
SCC, ×1000/mL	415	233	341	322	245	85	0.97	0.43	0.66	0.54

¹Values are least square means (n = 9).²Probability of a linear (L) or quadratic (Q) effect of Ca-tFA dose level.

treatments. Compared with the control cows, milk fat percentage and yield were decreased 3, 10, and 12% ($P < 0.03$) and 5, 11, and 15% ($P < 0.02$), respectively, in cows fed the Ca-tFA diets. Milk fat depression was greatest in cows fed the Ca-CLA diet, as milk fat percentage and yield were reduced by 25% ($P < 0.01$) and by 23% ($P < 0.01$), respectively, compared with the control.

Short- and medium-chain fatty acids were analyzed in a subset of milk samples (data not shown). Except for the butyric acid, milk yield of other fatty acids (6:0 to 12:0), synthesized in the mammary gland, tended to be lower in cows fed the Ca-tFA or Ca-CLA diets. Concentrations (g/100 g FAME) and yield (g/d) of individual fatty acids in milk fat are shown in Tables 5 and 6, respectively. Compared with the control cows, yield of both total saturated and *cis*-monounsaturated fatty acids was reduced by 25% ($P < 0.05$) and 26% ($P < 0.05$), respectively, by Ca-CLA (Table 6). Increasing amounts of Ca-tFA also decreased ($P < 0.01$) total yield of saturated fatty acids (SFA) (Table 6). This was largely due to the reduction in milk fat yield as concentrations of SFA were lower ($P < 0.02$) for C16:0 but numerically increased for C18:0 (Table 5). With the highest dose of Ca-tFA, yields of monounsaturated and SFA were reduced by 14 and 16%, respectively, again largely because of the overall reduction in milk fat yield. Secretion of PUFA was unchanged by the Ca-tFA diets, although it was numerically less, but not statistically different from the control, in milk of cows fed Ca-CLA.

Compared with the control, the concentration (Table 5) and yield (Table 6) of total CLA were greater in milk of cows fed the Ca-CLA ($P < 0.05$) and Ca-tFA ($P < 0.02$) diets. However, milk yield of CLA was not significantly different between the Ca-tFA and Ca-CLA treatments (Table 6). The CLA isomers in milk are presented in Table 7. Regardless of diet, *cis*-9, *trans*-11-18:2 was the predominant CLA isomer, varying from 68 to 76% of total CLA. Yields of *cis*-9, *trans*-11-18:2 ($P < 0.03$) and

trans-7, *cis*-9-18:2 ($P < 0.05$) were increased in milk with Ca-tFA supplementation and were higher than in milk of cows fed the control and the Ca-CLA diets. Compared with the controls, yield of *cis*-9, *trans*-11-18:2 was increased ($P < 0.05$) by the Ca-CLA supplementation, while *trans*-7, *cis*-9-18:2 was not different. As expected, the yield of *trans*-10, *cis*-12-18:2 ($P < 0.01$) was greatest in milk of cows fed the Ca-CLA diet, but the *trans*-8, *cis*-10-18:2 isomer was not found in measurable quantities in milk, even though it was present at 8.5% in the Ca-CLA supplement.

Total tFA percentage ($P < 0.01$) and yield ($P < 0.03$) were increased in milk of cows fed the Ca-tFA supplement (Tables 5 and 6). Except for the *trans*-15 and *trans*-16-18:1, which were minor components in the Ca-tFA isomer mixture, milk yields of the other *trans*-18:1 isomers (Table 8) were elevated with dietary addition of Ca-tFA. *Trans*-11-18:1 was the primary milk tFA isomer with each treatment and accounted for 24 to 29% of the total *trans*-18:1 isomers. Distribution of *trans*-18:1 isomers in milk of the cows fed Ca-CLA was similar to that observed in the control cows.

DISCUSSION

The Ca-tFA supplement used in this study was effective in decreasing milk fat yield without altering milk production. However, MFD in cows fed Ca-tFA was less pronounced compared with cows fed the Ca-CLA supplement. As expected, *trans*-10, *cis*-12-18:2, identified as the isomer responsible for MFD (Baumgard et al., 2000), was increased in milk fat of cows fed the Ca-CLA supplement. In contrast, the levels of this CLA isomer in milk of cows fed Ca-tFA diets did not increase with dose and were similar to the control value, indicating that the *trans*-10, *cis*-12-18:2 found in milk of these cows originated from the rumen (Griinari and Bauman, 1999; Corl et al., 2002; Piperova et al., 2002). These

Table 5. Concentration of fatty acids in milk of lactating cows fed diets supplemented with Ca salts of *trans* fatty acids (tFA) or conjugated linoleic acids (CLA).¹

							Contrast			
	Control	Ca-tFA			Ca-CLA	SEM	L ²	Q ²	CLA vs. control	CLA vs. tFA
		100 g	200 g	400 g	100 g					
	(g/100 g FAME)									
14:0	11.3	10.6	10.5	10.2	10.3	0.37	0.08	0.24	0.64	0.18
14:1	1.35	1.19	1.10	1.19	1.03	0.06	0.53	0.02	0.19	0.16
15:0	0.27	0.43	0.29	0.29	0.23	0.04	0.20	0.10	0.40	0.24
16:0	36.4	35.9	33.2	32.2	31.6	0.40	0.02	0.37	0.03	0.16
16:1 <i>cis</i>	2.08	1.70	1.84	1.75	1.34	0.20	0.21	0.23	0.05	0.07
17:0	0.59	0.59	0.61	0.59	0.69	0.02	0.62	0.79	0.93	0.73
17:1	0.24	0.18	0.22	0.20	0.27	0.02	0.27	0.87	0.31	0.69
18:0	11.9	13.0	13.4	13.2	12.8	0.70	0.12	0.73	0.18	0.65
18:1 <i>cis</i>	26.4	26.7	28.4	27.6	28.4	1.10	0.14	0.11	0.43	0.43
18:1 <i>trans</i>	2.88	3.99	4.31	5.37	3.02	0.20	0.01	0.37	0.73	0.01
18:2n-6	3.53	3.63	3.97	4.10	4.71	0.20	0.83	0.02	0.61	0.11
18:2i	0.26	0.37	0.28	0.51	0.56	0.07	0.68	0.93	0.18	0.06
18:3n-3	0.47	0.46	0.52	0.53	0.55	0.02	0.50	0.11	0.47	0.15
CLA	0.52	0.65	0.82	0.89	0.83	0.06	0.10	0.03	0.05	0.61
≥C20	0.67	0.61	0.55	0.62	0.79	0.04	0.80	0.36	0.33	0.76

¹Values for fatty acids are least square means (n = 9). Some fatty acids (≥C:14) which represent <3% of the total fatty acid methyl esters (FAME) were not included. 18:2i = Nonconjugated dienes with one or two *trans* double bonds.

²Probability of a linear (L) and quadratic (Q) effect of dose level.

results were consistent with our finding that the Ca-tFA supplement lacked any detectable *trans*-10, *cis*-12-18:2 and strongly suggest that some components other than *trans*-10, *cis*-12-18:2 was involved in the MFD observed in cows fed the Ca-tFA supplement.

In addition to the *trans*-10, *cis*-12-18:2, dietary MFD has been associated with increased levels of tFA and, particularly, with an altered isomer pattern in which the *trans*-10-18:1 isomer was preferentially increased (Grinari et al., 1998; Newbold et al., 1998; Piperova,

Table 6. Yield of fatty acids in milk of lactating cows fed diets supplemented with Ca salts of *trans* fatty acids (tFA) or conjugated linoleic acids (CLA).¹

	Contrast									
	Control	Ca-tFA			Ca-CLA		Contrast			
		100 g	200 g	400 g	100 g	SEM	L ²	Q ²	CLA vs. control	CLA vs. tFA
	(g/d)									
14:0	136.9	127.2	117.8	115.2	100.9	4.3	0.22	0.01	0.03	0.18
14:1	16.6	14.5	13.1	12.9	9.2	1.3	0.01	0.02	0.05	0.06
15:0	13.5	15.8	13.2	12.3	12.1	0.5	0.10	0.01	0.24	0.02
16:0	436.3	398.9	366.1	350.5	321.2	11.1	0.63	0.01	0.34	0.06
16:1 <i>cis</i>	25.8	20.7	20.9	19.1	11.6	0.8	0.21	0.03	0.05	0.05
17:0	7.3	7.1	6.9	6.3	5.2	0.4	0.10	0.02	0.08	0.17
17:1	2.9	2.1	2.5	2.1	1.5	0.2	0.17	0.13	0.12	0.15
18:0	145.5	156.8	153.2	139.1	112.4	5.1	0.02	0.03	0.05	0.19
18:1 <i>cis</i>	323.7	345.1	344.7	286.5	258.3	19.0	0.04	0.11	0.43	0.08
18:1 <i>trans</i>	36.1	45.5	48.3	56.9	31.2	3.1	0.01	0.03	0.07	0.01
18:2n-6	44.1	44.5	44.6	42.4	40.9	3.1	0.18	0.29	0.25	0.37
18:2i	3.2	4.5	4.1	5.4	3.4	0.8	0.10	0.11	0.22	0.08
18:3n-3	5.9	5.6	6.0	5.6	4.3	0.5	0.06	0.21	0.07	0.66
CLA	6.5	8.0	8.5	8.9	8.1	0.3	0.02	0.22	0.05	0.61
≥C20	8.4	6.7	6.1	6.3	5.6	0.8	0.67	0.03	0.06	0.29
<i>cis</i> -mono FA	371.2	381.3	379.0	319.6	281.6	13.2	0.04	0.06	0.05	0.07
PUFA	53.1	54.6	53.9	53.3	48.6	2.3	0.15	0.25	0.15	0.93
SFA	740.5	706.6	655.6	624.4	551.8	22.3	0.19	0.01	0.05	0.05

¹Values for fatty acids are least square means (n = 9). Some fatty acids (≥C:14) which represent <3% of the total fatty acid methyl esters (FAME) were not included. 18:2i = Nonconjugated dienes with one or two *trans* double bonds. PUFA = Polyunsaturated FA; SFA = saturated FA.

²Probability of a linear (L) and quadratic (Q) effect of dose level.

Table 7. Conjugated linoleic acid isomers in milk fat of lactating cows fed diets supplemented with Ca salts of *trans* fatty acids (tFA) or conjugated linoleic acids (CLA).¹

	Control	Ca-tFA			Ca-CLA	SEM	Contrast			
		100 g	200 g	400 g	100 g		L ²	Q ²	CLA vs. control	CLA vs. tFA
CLA isomers		(g/100 g FAME)								
t-7, c-9	11.25	12.83	14.19	14.43	9.10	0.52	0.01	0.39	0.01	0.01
c-9, t-11	76.82	75.49	75.83	76.06	67.94	0.97	0.01	0.01	0.01	0.01
t-10, c-12	1.32	1.12	0.98	0.81	3.48	0.16	0.01	0.01	0.01	0.01
c-11, t-13	0.46	0.61	0.55	0.55	3.40	0.11	0.01	0.01	0.01	0.01
<i>trans/trans</i>	8.41	7.91	7.41	7.01	11.11	0.78	0.01	0.09	0.02	0.03
<i>cis/cis</i>	1.64	1.66	1.02	1.06	4.50	0.29	0.06	0.64	0.02	0.02
		(g/d)								
t-7, c-9	0.82	1.07	1.29	1.30	0.66	0.09	0.05	0.20	0.25	0.05
c-9, t-11	4.99	5.95	6.76	6.80	5.50	0.34	0.03	0.22	0.05	0.05
t-10, c-12	0.09	0.09	0.08	0.07	0.28	0.03	0.07	0.11	0.01	0.01
c-11, t-13	0.03	0.05	0.05	0.05	0.19	0.01	0.01	0.01	0.01	0.01
<i>trans/trans</i>	0.56	0.63	0.67	0.63	0.90	0.02	0.05	0.06	0.03	0.07
<i>cis/cis</i>	0.11	0.13	0.09	0.10	0.36	0.02	0.03	0.05	0.05	0.05

¹Values for fatty acids are least square means (n = 9).²Probability of a linear (L) or quadratic (Q) effect of Ca-tFA dose level.

et al., 2000; Peterson et al., 2003). By design, the Ca-tFA supplement provided a high proportion of *trans*-10-18:1 and elevated amounts of this isomer were incorporated into the milk fat of cows fed this supplement. It is tempting to ascribe the MFD observed in cows fed the Ca-tFA diets to the *trans*-10-18:1 isomer. Nevertheless, the Ca-tFA supplement contained several tFA iso-

mers in relatively high proportions, and, in addition to the *trans*-10-18:1, the yields of *trans*-9, *trans*-11, and *trans*-12-18:1 were consistently increased in milk of cows fed increasing doses of the Ca-tFA supplement. It is unlikely that these tFA isomers are independently involved in MFD, as others have shown that *trans*-9-18:1 (Rindsig and Schultz, 1974; Newbold et al., 1998),

Table 8. *Trans*-18:1 fatty acid isomers in milk fat of lactating cows fed diets supplemented with Ca salts of *trans* fatty acids (tFA) and conjugated linoleic acids (CLA).¹

	Control	Ca-tFA			Ca-CLA	SEM	Contrast			
		100 g	200 g	400 g	100 g		L ²	Q ²	CLA vs. control	CLA vs. tFA
<i>Trans</i> -18:1 isomers		(g/100 g tFA)								
4+5	0.92	1.57	1.19	1.36	1.04	0.13	0.17	0.15	0.50	0.03
6+7+8	2.88	3.57	5.15	3.63	2.45	0.59	0.33	0.03	0.63	0.02
9	3.70	5.76	7.86	6.71	3.56	0.83	0.01	0.01	0.89	0.01
10	13.10	14.49	18.20	19.20	12.65	0.74	0.01	0.04	0.65	0.01
11	29.30	26.83	25.50	24.45	27.64	1.10	0.03	0.01	0.23	0.03
12	12.69	12.22	12.76	12.25	13.29	0.50	0.88	0.72	0.41	0.11
13+14	22.68	20.95	19.00	20.73	24.08	0.36	0.01	0.01	0.02	0.01
15	7.50	7.47	5.74	5.75	7.54	0.19	0.01	0.02	0.89	0.01
16	7.14	7.14	5.60	5.52	7.75	0.37	0.03	0.77	0.26	0.02
		(g/d)								
4+5	0.33	0.71	0.57	0.77	0.32	0.09	0.02	0.10	0.06	0.05
6+7+8	1.04	1.62	2.49	2.07	0.70	0.34	0.05	0.96	0.11	0.01
9	1.34	2.62	3.80	4.43	1.11	0.63	0.05	0.81	0.15	0.05
10	4.74	6.59	8.79	10.92	3.95	0.62	0.01	0.35	0.17	0.01
11	10.60	12.21	12.32	13.91	8.62	0.82	0.05	0.01	0.07	0.04
12	4.58	5.56	6.16	6.97	4.15	0.47	0.02	0.42	0.21	0.05
13+14	8.19	9.53	9.18	11.80	7.51	0.70	0.03	0.07	0.16	0.06
15	2.71	3.40	2.77	3.27	2.35	0.23	0.07	0.05	0.16	0.07
16	2.58	3.25	2.68	3.37	2.42	0.22	0.08	0.06	0.07	0.06

¹Values for fatty acids are least square means (n = 9).²Probability of a linear (L) or quadratic (Q) effect of Ca-tFA dose level.

trans-11-18:1, and *trans*-12-18:1 (Newbold et al., 1998; Griinari et al., 2000) were ineffective in reducing milk fat in lactating cows.

Other tFA of interest were *trans*-6+7+8-18:1 and *trans*-13+14-18:1, which were also present in the Ca-tFA supplement and transferred in moderate amounts to the milk of cows fed this supplement. Increased concentrations of *trans*-6+7+8-18:1 and *trans*-13+14-18:1 have been reported previously in milk of cows fed MFD diets (Griinari et al., 1998; Piperova et al., 2000, 2002; Precht et al., 2002; Peterson et al., 2003). However, the increase in *trans*-6+7+8-18:1 and *trans*-13+14-18:1 reported by others (Griinari et al., 1998; Loor and Herbein, 2003) was not always consistent with reduction in milk fat. Moreover, comparisons among the Ca-tFA treatments herein make it unlikely that these 2 groups of tFA are involved in MFD. Although the increase in *trans*-6+7+8-18:1 (g/d) over the control value was greatest for the 200-g Ca-tFA diet, this treatment did not result in the greatest decrease in milk fat yield. Conversely, the yield of *trans*-13+14-18:1 was lowest in cows fed the 200 g of Ca-tFA despite the moderate MFD observed with this dose. In other previous work, reduction in milk fat was not achieved in cows fed diets supplemented with 3 different fats (Kalscheur et al., 1997b), even though subsequent analysis of the milk fat revealed elevated levels of *trans*-6+7+8-18:1 (0.53, 0.79, 0.82, and 0.77 g/d) and *trans*-13+14-18:1 (9.76, 13.45, 18.22, and 22.8 g/d) respectively, for cows fed the control and the fat-supplemented diets (L. S. Piperova, 2001, unpublished data). In view of the previous discussion, if a *trans*-18:1 isomer is involved in MFD, the *trans*-10-18:1 seems to be the most likely candidate.

In this study, sources of *trans*-10-18:1 in milk of cows fed the Ca-tFA diet included those arising from both rumen biohydrogenation of PUFA and Ca-tFA supplementation. Intake of *trans*-10-18:1 was 8.4, 16.8, and 33.4 g/d, respectively, with the 100-, 200-, and 400-g Ca-tFA diets. If rumen biohydrogenation was the only source contributing to *trans*-10-18:1 in milk of the control cows, then the amount derived from the Ca-tFA was calculated to be 1.8, 4.0, and 6.2 g/d, respectively, for the 100-, 200-, and 400-g Ca-tFA diets. These increases in *trans*-10-18:1 were not as large as those observed previously during dietary induced MFD (Griinari et al., 1998; Piperova et al., 2000; Peterson et al., 2003) and corresponded to 21, 23, and 19% apparent transfer efficiencies with increasing doses of Ca-tFA. Partial hydrogenation of the Ca-tFA supplement might account for at least some of the reduced tFA in milk. Wu et al. (1991) showed that protection of unsaturated fatty acids from rumen biohydrogenation is incomplete and that 57% of dietary unsaturated fatty acids, fed as Ca salts, were hydrogenated by rumen microorganisms.

If we assume a biohydrogenation value of 57% for *trans*-10-18:1, as Ca salt, amounts of this isomer available for transfer to milk would have been 3.8, 7.7, and 15.4 g/d, respectively, for cows fed the 100-, 200-, and 400-g Ca-tFA diets. Thus, the increased yields of *trans*-10-18:1 in milk would reflect transfer efficiencies from 40 to 52%. These values are in general agreement with transfer efficiencies calculated for *trans*-10-18:1 from data comparing duodenal contents and milk fat in cows fed a MFD diet (Piperova et al., 2002).

The apparent transfer efficiency of *trans*-10, *cis*-12-18:2 in this study (5%) was somewhat higher than the 2.4 to 4.4% and 3.4 % reported by Giesy et al. (2002) and Perfield II et al. (2002), respectively, for this isomer in milk of cows fed greater doses of rumen-protected CLA. The amount of *trans*-10, *cis*-12-18:2 in milk of the cows fed Ca-CLA (0.28 g/d) was similar to the 0.30 g/d observed in the study by Perfield II et al. (2002), resulting in a 23% decrease in milk fat yield in both studies. The apparent transfer efficiency of *trans*-10, *cis*-12-18:2 from Ca-CLA supplements was lower compared with that observed (10 to 21%) for this isomer during abomasal infusion of CLA supplements (Chouinard et al., 1999a, b). Nevertheless, the degree of milk fat reduction reported herein (23%) and by Perfield II et al. (2002) was almost identical (24%) to the MFD achieved in response to higher levels of *trans*-10, *cis*-12-18:2 (0.86 g/d) in milk provided by abomasal infusion of pure *trans*-10, *cis*-12 CLA (Baumgard et al., 2001). Thus, the MFD caused by the Ca-CLA diet was greater than expected based on the concentration of *trans*-10, *cis*-12-18:2 in milk.

Discrepancies between concentrations of *trans*-10, *cis*-12-18:2 in milk and degree of milk fat reduction have been observed previously (Loor et al., 2002; Peterson et al., 2003) during dietary-induced MFD, suggesting that other substances may also be involved. In this experiment, the *cis*-9, *trans*-11-18:2; the *cis*-11, *trans*-13-18:2; the *trans*, *trans* and the *cis*, *cis* CLA isomers were also increased in milk by the Ca-CLA supplement. However, it has been demonstrated that abomasal infusion of *cis*-9, *trans*-11-18:2 (Baumgard et al., 2000) or *trans*-8, *cis*-10 and *cis*-11, *trans*-13-18:2 (Perfield II et al., 2004) had no effect on milk fat synthesis, and Loor and Herbein (2003) did not observe MFD in feeding experiments in which the *trans*, *trans* and the *cis*, *cis* CLA isomers in milk were elevated. Perhaps, as Bauman and Griinari (2001, 2003) have suggested, other unidentified intermediates, produced in the rumen, may also be involved in MFD. Further research is required to investigate these discrepancies.

In this study, milk concentrations and yield of CLA were not different among cows fed the Ca-supplemented diets; however, changes in the CLA isomer pro-

file were observed. It has been reported that formation of CLA in the rumen cannot account for the concentrations of CLA in milk (Piperova et al., 2002) and that Δ^9 -desaturation of ruminally derived *trans*-18:1 precursors is the primary source of *cis*-9 containing CLA in milk (Griinari et al., 2000; Corl et al., 2001, 2002). The increase in yield of *cis*-9 containing CLA observed in milk of cows fed Ca-tFA diets was in agreement with these findings. Compared with the control, milk secretion of *trans*-11-18:1 was increased by 15, 16, and 31%, respectively, with the Ca-tFA diets and resulted in a 19% (100 g Ca-tFA), 35% (200 g Ca-tFA), and 36% (400 g Ca-tFA) increase milk yield of *cis*-9, *trans*-11-18:2. Griinari et al. (2000) have shown that abomasal infusion of *trans*-11-18:1 in lactating cows resulted in a 31% increase in the yield of *cis*-9, *trans*-11-18:2, accounting for 12% of the infused *trans* monoene. Availability of *trans*-7-18:1 in milk was not determined in this experiment; however, milk yield of *trans*-7, *cis*-9-18:2 was increased by 30, 57, and 58%, respectively, with increasing doses of Ca-tFA, suggesting that a sufficient amount of *trans*-7-18:1 was delivered to the mammary gland to account for the increase.

The *cis*-9, *trans*-11-18:2 in milk of cows fed Ca-CLA originated from 3 sources: biohydrogenation of PUFA in the rumen, endogenous synthesis via Δ^9 -desaturation of *trans*-11-18:1, and the amount transferred from the supplement. Under normal rumen conditions, the portion of *cis*-9, *trans*-11-18:2 that escapes biohydrogenation is minor (Piperova et al., 2002; Corl et al., 2002). Thus, we assumed that all of the milk *cis*-9, *trans*-11-18:2 CLA was derived from the supplement and from endogenous synthesis. Furthermore, the amount derived from endogenous synthesis can be estimated from the quantity of this isomer in control milk, if Δ^9 -desaturation was not appreciably altered by the Ca-CLA supplement. Indeed, this appeared to be the case when we examined the values for the *trans*-7, *cis*-9-18:2, an isomer not present in the Ca-CLA supplement and known to be almost exclusively produced via Δ^9 -desaturation of *trans*-7-18:1 (Piperova et al., 2002; Corl et al., 2002). Because yields of *trans*-7, *cis*-9-18:2 and *trans*-6+7+8-18:1 in milk of cows fed Ca-CLA were not significantly different from the control values, we believe that Δ^9 -desaturation in the mammary gland was not altered by the Ca-CLA supplementation. This is consistent with data reported by Giesy et al. (2002) and Perfield II et al. (2002), showing that ratios of fatty acids representing product/substrate for Δ^9 -desaturase were not affected in milk of cows fed doses of Ca salts of CLA higher than those used in the present study. Hence, the apparent transfer efficiency of *cis*-9, *trans*-11-18:2 from the supplement to milk (11%) was calculated assuming that the difference in *cis*-9, *trans*-11-

18:2, compared with the control, was derived from the Ca-CLA supplement. A similar transfer efficiency (9 to 10%) was reported for *cis*-9, *trans*-11-18:2 by Giesy et al. (2002) in lactating cows fed increasing doses of Ca-CLA-60. These transfer efficiencies were lower than the 22.5% observed for this isomer during abomasal infusion of a mixture of CLA isomers (Chouinard et al., 1999a). As suggested by Giesy et al. (2002), site of supplementation may be an important factor in the efficiency of CLA transfer into milk.

CONCLUSIONS

We have shown that dietary Ca-tFA supplementation can reduce milk fat content and yield; however, the MFD was less pronounced, compared with Ca-CLA supplementation. The concentration and yield of *cis*-9 containing CLA were highest in milk of cows fed the Ca-tFA supplement, which provided the *trans*-7 and *trans*-11-18:1 precursors for the endogenous synthesis of CLA in the mammary gland. The decrease in milk fat in cows fed Ca-CLA was associated with the greater level of *trans*-10, *cis*-12-18:2 in milk. In contrast, concentrations of *trans*-10, *cis*-12-18:2 were not altered by supplementation with Ca-tFA, whereas the levels of *trans*-18:1, including the *trans*-10 isomer, were elevated in milk of cows fed the tFA supplements. These results provide evidence that an increase in *trans*-10, *cis*-12-18:2 is not required for MFD and suggest that other components, including the *trans*-10-18:1, could also be involved.

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