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Journal of Nutritional Biochemistry 12 (2001) 622–630

THE JOURNAL OF Nutritional Biochemistry

The role of Δ^9-desaturase in the production of *cis*-9, *trans*-11 CLA☆

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Received 28 February, 2001; received in revised form 5 June, 2001; accepted 3 July, 2001

Abstract

Biomedical studies with animal models have demonstrated that *cis*-9, *trans*-11 conjugated linoleic acid (CLA), the predominant isomer found in milk fat from dairy cows, has anticarcinogenic effects. We recently demonstrated endogenous synthesis of *cis*-9, *trans*-11 CLA from ruminally derived *trans*-11 C18:1 by Δ^9-desaturase in lactating dairy cows. The present study further examined endogenous synthesis of *cis*-9, *trans*-11 CLA and quantified its importance by increasing substrate supply using partially hydrogenated vegetable oil (PHVO) as a source of *trans*-11 C18:1 and blocking endogenous synthesis using sterculic oil (SO) as a source of cyclopropene fatty acids which specifically inhibit Δ^9-desaturase. Four cows were abomasally infused with 1) control, 2) PHVO, 3) SO, and 4) PHVO+SO in a 4 x 4 Latin square design. With infusion of PHVO, *cis*-9, *trans*-11 CLA was increased by 17% in milk fat. Consistent with inhibition of desaturase, SO treatments increased milk fat ratios for the fatty acid pairs effected by Δ^9-desaturase, C14:0/*cis*-9 C14:1, C16:0/*cis*-9 C16:1, and C18:0/*cis*-9 C18:1. The role of endogenous synthesis of CLA was evident from the 60–65% reduction in *cis*-9, *trans*-11 CLA which occurred in milk fat with SO treatments. *cis*-9 C14:1 originates from desaturation of C14:0 by Δ^9-desaturase and can be used to estimate the extent of SO inhibition of Δ^9-desaturase. When this correction factor was applied, endogenous synthesis was estimated to account for 78% of the total *cis*-9, *trans*-11 CLA in milk fat. Thus, endogenous synthesis was the major source of *cis*-9, *trans*-11 CLA in milk fat of lactating cows.

Keywords: Δ^9-Desaturase; Trans fatty acid; Conjugated linoleic acid; Milk fat; Sterculic acid; Cyclopropene fatty acids

1. Introduction

Functional foods contain dietary components that have beneficial properties beyond their traditional nutrient value [1]. Conjugated linoleic acid (CLA) represents a functional food component found in dairy products. CLA is a collective term for isomers of linoleic acid with conjugated double bonds in several positions and conformations. The predominant CLA found in milk fat, the *cis*-9, *trans*-11 isomer, has been shown to be anticarcinogenic in animal models [2]. Butter containing significantly increased CLA concentrations has also been shown to be anticarcinogenic in a rat mammary cancer model [3].

Ruminal biohydrogenation of linoleic acid produces *cis*-9, *trans*-11 CLA as the first intermediate and it has been generally assumed that the CLA in milk fat had escaped complete biohydrogenation in the rumen [4]. However, we recently demonstrated endogenous synthesis of *cis*-9, *trans*-11 CLA in lactating dairy cows [5]. This pathway involves Δ^9-desaturase and the desaturation of ruminally derived *trans*-11 C18:1, the second intermediate of linoleic acid biohydrogenation. Others have also shown an endogenous synthesis of *cis*-9, *trans*-11 CLA in mice [6], rats [3], and humans [7,8] based on the increase in CLA observed with diets supplemented with *trans*-11 C18:1. The final step in rumen biohydrogenation of linoleic acid is the hydrogenation of *trans*-11 C18:1 to C18:0.

Our objective was to further examine endogenous synthesis of *cis*-9, *trans*-11 CLA by lactating dairy cows and quantify its importance by enhancing and inhibiting endog-

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☆Presented at the annual meeting of the American Dairy Science Association in 2000; Corl, B.A., Baumgard, L.H., Dwyer, D.A., Griinari, J.M., Phillips, B.S., and Bauman, D.E., The role of Δ^9-desaturase in the production of *cis*-9, *trans*-11 CLA and other Δ^9-desaturated fatty acids in milk fat. J. Dairy Sci. 83(suppl. 1):164. Baltimore, MD.

0955-2863/01/$ – see front matter
PII: S0955-2863(01)00180-2

Table 1
Ingredient and chemical composition of the experimental diet

Composition	Content
Ingredient (g/100 g dry matter)	
Chopped alfalfa hay	45.08
Ground corn	22.82
Soybean meal	7.04
Extruded soybeans	8.36
Citrus pulp	9.89
Whole cottonseed	4.40
Limestone	0.71
Dicalcium phosphate	0.69
Sodium bicarbonate	0.53
Magnesium oxide	0.16
Mineral-vitamin mix[1]	0.33
Chemical Analysis[2]	
Dry matter (g/100 g)	90.1
Crude protein (g/100 g dry matter)	16.4
Crude fat (g/100 g dry matter)	4.73
Neutral detergent fiber (g/100 g dry matter)	41.2
Acid detergent fiber (g/100 g dry matter)	31.2
Net energy for lactation (MJ/kg dry matter)	6.63

[1] Contained 385 g NaCl and 615 g of a trace mineral and vitamin mix per kg of supplement. Trace mineral and vitamin mix contained (g/kg mix) Mn, 1.1; Zn 1.4; Fe, 0.50; Cu, 0.25; I, 0.027; Co, 0.024; Se, 0.007; retinyl acetate, 0.258; cholecalciferol, 0.007; and *dl*-α-tocopheryl acetate, 2.56.
[2] Analyses were by Dairy One, Ithaca, NY.

enous synthesis. For the former we increased the substrate for endogenous synthesis using partially hydrogenated vegetable oil (PHVO) as a source of *trans*-11 C18:1. For the latter, we blocked endogenous synthesis using sterculic oil (SO) as a source of cyclopropene fatty acids which specifically inhibit Δ^9-desaturase [9].

2. Materials and methods

2.1. Animals and experimental design

All procedures using animals were approved by the Cornell University Institutional Animal Care and Use Committee. Four lactating multiparous, Holstein cows (115 ± 9 days in milk, mean ± SD) fitted with rumen cannulae were fed a total mixed ration (Table 1) formulated using the Cornell Net Carbohydrate and Protein System [10] to meet or exceed requirements [11]. Cows were fed *ad libitum* with fresh feed offered at 0600 and 1800 daily.

The experimental design was a 4 x 4 Latin square experiment with four day treatment periods and a six day interval between periods. Treatments were 1) control, 2) PHVO, 3) SO, and 4) PHVO+SO. The partially hydrogenated vegetable oil, obtained from Raisio Chemicals (Raisio, Finland), was solid at room temperature. For infusion (250 g/day), it was melted, infused, and then chased with 180 mL warm water (60°C) to evacuate the infusion line of any remaining oil. Sterculic oil was obtained from the seeds of the *Sterculia foetida* tree. Trees under private ownership in India were identified and seeds harvested and imported by permit from USDA Animal and Plant Health Inspection Service (APHIS). Seed hulls were removed and the meat was then crushed and extracted using diethyl ether [12]. The extracted oil represented approximately 17% of the seeds and 41% of the seed meat (weight basis; Table 2). Sterculic oil was prepared as an emulsion in skim milk to provide sufficient volume for accurate infusion. Emulsions were prepared as previously described using a microfluidizer [13] and the target sterculic oil concentration was 2%. The actual concentration was 1.76 ± 0.15% (mean ± SD) so that the 500 mL/d delivered approximately 8.8 g/day of sterculic oil. An equal volume of skim milk was infused for the control treatment. Treatment solutions were infused into the abomasum via polyvinyl chloride tubing (0.5 cm i.d.), which passed through the rumen cannula and sulcus omasi into the abomasum [14]. One fourth of the daily dose was infused every 6 hr.

Table 2
Components and fatty acid composition of *Sterculia foetida* seeds

Variable	Content
Seed components (g/100 g total seeds)	
Hull	57.6
Meat	42.4
Pulp	25.2
Oil	17.2
Oil composition (g/100 g fatty acids)	
16:0	22.95
18:1, cyclo[1]	6.33
18:0	1.87
18:1, *cis*-9	5.08
18:2, *cis*-9, *cis*-12	5.03
19:1, cyclo[1]	55.86
Other	2.88

[1] cyclo = presence of cyclopropene ring. C18:1, cyclo is 7-(2-octyl-1-cyclopropenyl) heptanoic acid and C19:1, cyclo is 8-(2-octyl-1-cyclopropenyl) octanoic acid.

Cows were milked twice daily at 0600 and 1800. At each milking, yield was recorded and milk sampled. One aliquot was stored at 4°C with a preservative tablet (bronopol tablet; D & F Control System, San Ramon, CA) until infrared analysis for fat and protein content (Dairy One, Ithaca, NY). A second aliquot was stored at -20°C until analyzed for fatty acid composition. Blood samples were taken from the coccygeal vein after the 1800 milking on days 0, 3 and 4 of infusion. Sodium heparin (100 U/ml of blood) was used to prevent coagulation. Plasma was harvested (2,300 x *g*, 15 min at 4°C) and stored at -20°C until analyzed for fatty acid composition.

2.2. Analyses

For fatty acid analysis of milk, lipids were extracted using the method of Hara and Radin [15] as modified by Chouinard et al. [14] Fatty acid methyl esters were prepared

by transesterification with sodium methoxide [16] as adapted by Chouinard et al. [13] The fatty acids in SO were transmethylated by the same procedure as used for milk fat.

For fatty acid analysis of plasma, lipids were extracted using the method of Hara and Radin [15] with modifications. To 2.0 mL plasma was added 3.0 mL of hexane-isopropanol solution (3:2, v/v) followed by 2.0 mL sodium sulfate solution (67 g/L). The upper phase was removed and dried over 1.0 g anhydrous sodium sulfate. The solution was then transferred again and taken to dryness under a continuous stream of nitrogen. Plasma lipids were transmethylated with sodium methoxide according to the method of Christie [16]. Briefly, hexane (0.5 mL) was added to the extracted plasma lipids followed by 40 μL methyl acetate. After the mixture was vortexed, 40 μl methylation reagent (1.75 mL methanol: 0.4 mL of 5.4 M sodium methoxide) was added. The mixture was vortexed and allowed to react at room temperature for 24 hr, then 60 μL of termination reagent (1.0 g oxalic acid in 30 mL diethyl ether) were added. A few grains of calcium chloride were added to remove methanol. The solution containing the fatty acid methyl esters was removed for subsequent analysis.

Fatty acids in PHVO were methylated using trimethylsilyldiazomethane according to Hashimoto et al. [17] with modifications. To the fatty acids (40 mg), 1.6 mL hexane and 0.4 mL methanol were added. One mL of 30 mM trimethylsilyldiazomethane in hexane was added and allowed to stand at room temperature for 30 min. The reaction was stopped with 5 drops acetic acid. After the addition of 5.0 mL water, the hexane phase was dried over 1.0 g sodium sulfate and then transferred again and taken to dryness under a continuous stream of nitrogen. The fatty acid methyl esters were then dissolved in 2.0 mL hexane for analysis.

Fatty acid methyl esters from milk fat and sterculic oil were quantified by gas chromatography (Hewlett Packard GCD system HP G1800 A; Avondale, PA) equipped with a CP-Sil 88 column (100 m x 0.25 mm i.d. with 0.20 μm film thickness; Chrompack, The Netherlands). The analyses involved a programmed run with temperature ramps. The oven temperature was initially 50°C for 1 min then ramped to 160°C at 5°C/min and held for 42 min. The temperature was then ramped again at 5°C/min to 190°C and held for 22 min. Injector and detector temperatures were maintained at 250°C, the helium carrier gas flow rate was 1 ml/min, and the split ratio was 100:1.

Fatty acid methyl esters from plasma and PHVO were quantified by gas chromatography (Hewlett Packard GC system 6890+) equipped with a flame ionization detector and equipped with a SP-2560 capillary column (100 m $\times$ 0.25 mm i.d. with 0.2 μm film thickness; Supelco Inc., Bellefonte, PA). The oven temperature was initially 160°C and held for 28 min. The oven temperature was then ramped at 5^{o}/min to 220^{o} and held for 25 min. Inlet and detector temperature were maintained at 250°C and the split ratio was 100:1. The hydrogen carrier gas flow rate was 1 mL/min. Hydrogen flow to the detector was 25 ml/min, air flow was 400 ml/min, and the nitrogen make-up gas flow was 45 ml/min.

Table 3
Fatty acid composition of the partially hydrogenated vegetable oil

Fatty acid	Content (g/100 g fatty acids)
12:0	1.18
14:0	0.61
16:0	11.70
18:0	5.61
18:1, *trans*-6 to 8	3.61
18:1, *trans*-9	12.80
18:1, *trans*-10	12.15
18:1, *trans*-11	9.40
18:1, *trans*-12	6.09
Other	36.85

For both gas chromatography systems, each peak was identified and quantified using pure methyl ester standards (Nu Chek Prep, Elysian, MN). A butter reference standard (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to determine recoveries and correction factors for individual fatty acids.

2.3. Statistics

Data were statistically analyzed as a 4 x 4 Latin square using the PROC Mixed procedure of SAS [18]. For milk fatty acids, the model included treatment, period, and cow. The model for plasma fatty acids included treatment, period, and cow with day 0 as a covariate. Significant differences between treatments were determined using a *t* test.

3. Results

Dietary supplements of PHVO and SO were abomasally infused as a convenient experimental method to avoid ruminal biohydrogenation. PHVO provided a supply of *trans*-11 C18:1 as substrate for Δ^9-desaturase synthesis of *cis*-9, *trans*-11 CLA. The PHVO supplement contained approximately 44% *trans* fatty acids with *trans*-11 C18:1 accounting for 9.4% of the total fatty acids (Table 3). Thus, the infusion supplied approximately 23.5 g/day of *trans*-11 C18:1. The ability of SO to block endogenous synthesis of CLA via inhibition of Δ^9-desaturase was due to the cyclopropene fatty acids, sterculic acid (C19:1) and malvalic acid (C18:1). In the present study, these two cyclopropene fatty acids, comprised 62.2% of the sterculic oil (Table 2).

Milk yield and dry matter intake were unaffected by experimental treatments (Table 4). Similarly, milk protein yield was not affected, although small differences in milk protein content occurred (Table 4). In contrast, experimental treatments modestly reduced milk fat yield and content (8–17%).

Table 4
Performance of lactating dairy cows during abomasal infusion of partially hydrogenated vegetable oil (PHVO) and sterculic oil (SO)

Variable	Treatment				SEM	P^1
	Control	PHVO	SO	PHVO + SO		
Dry Matter Intake (kg/d)	27.3	25.1	26.3	26.7	0.6	0.10
Milk (kg/d)	39.8	37.8	38.6	38.4	0.7	0.27
Milk Protein						
kg/d	1.08	1.04	1.07	1.08	0.02	0.44
%	2.71[b]	2.75[bc]	2.78[ac]	2.83[a]	0.01	0.01
Milk Fat						
kg/d	1.33[a]	1.11[b]	1.17[b]	1.25[ab]	0.05	0.03
%	3.32[a]	2.90[b]	3.03[bc]	3.24[ac]	0.08	0.01

[1] Statistical probability of treatment differences. Values represent means for days 3 and 4 of the treatment period and means within row with different superscripts differ ($P < 0.05$).

Effects of treatment on the fatty acid composition of plasma and milk fat are presented in Tables 5 and 6. PHVO treatment resulted in the expected increase in *trans*-C18:1 fatty acids in plasma and milk fat. Specifically, *trans*-11 C18:1 content was increased by 46% in plasma and 39% in milk fat. Consistent with endogenous synthesis of CLA, PHVO infusion also increased *cis*-9, *trans*-11 CLA, and this was especially apparent in milk fat. Treatment with SO inhibited Δ^9-desaturase as evident by changes in fatty acid composition. For milk fatty acids, decreases of 84%, 59% and 46% were observed for *cis*-9 C14:1, *cis*-9 C16:1, and *cis*-9 C18:1, respectively. Consistent with inhibition of desaturase, SO treatment increased milk fat ratios for the fatty acid pairs effected by Δ^9-desaturase, C14:0/*cis*-9 C14:1, C16:0/*cis*-9 C16:1, and C18:0/*cis*-9 C18:1. Plasma concentrations of *cis*-9 C16:1 and *cis*-9 C18:1 were also reduced with SO treatment, although to a lesser extent. The role of endogenous synthesis of CLA was evident from the marked reduction in *cis*-9, *trans*-11 CLA and the 18% increase in *trans*-11 C18:1 which occurred in milk fat with SO treatment. The temporal pattern for *cis*-9, *trans*-11 CLA and *trans*-11 C18:1 further illustrated the reciprocal changes in these fatty acids when SO was infused (Fig. 1).

The PHVO+SO treatment gave mixed results in terms of changes in plasma and milk fatty acids. *trans* fatty acids were increased in a manner similar to the PHVO treatment whereas fatty acids related to Δ^9-desaturase were decreased similar to the SO treatment (Tables 5 and 6). However, there

Table 5
Composition of plasma lipids during abomasal infusion of partially hydrogenated vegetable oil (PHVO) and sterculic oil (SO) in lactating dairy cows

Fatty Acid	Treatment				SEM	P^1
	Control	PHVO	SO	PHVO + SO		
Content (g/100 g fatty acids)						
15:0	0.51[b]	0.53[a]	0.53[a]	0.54[a]	<0.01	0.01
16:0	8.42[b]	8.78[a]	8.33[b]	8.28[b]	0.11	0.02
16:1, *cis*-9	0.23[a]	0.23[a]	0.21[b]	0.21[b]	<0.01	0.01
17:0	0.62[a]	0.58[b]	0.62[a]	0.58[b]	0.01	0.02
18:0	16.08[a]	15.30[b]	16.22[a]	14.98[b]	0.30	0.05
18:1, *trans*-6 to 8	0.07[b]	0.12[a]	0.08[b]	0.12[a]	<0.01	0.01
18:1, *trans*-9	0.07[c]	0.31[a]	0.07[c]	0.29[b]	<0.01	0.01
18:1, *trans*-10	0.11[b]	0.24[a]	0.11[b]	0.23[a]	<0.01	0.01
18:1, *trans*-11	0.50[b]	0.73[a]	0.50[b]	0.73[a]	0.01	0.01
18:1, *trans*-12	0.36[b]	0.51[a]	0.34[b]	0.49[a]	<0.01	0.01
18:1, *cis*-9	2.93[b]	3.26[a]	2.68[d]	2.81[c]	0.04	0.01
18:2, *cis*-9, *cis*-12	46.34	45.61	46.67	46.94	0.50	0.29
18:3, *cis*-9, *cis*-12, *cis*-15	2.94	2.89	3.03	3.02	0.05	0.14
cis-9, *trans*-11 CLA	0.07[b]	0.08[a]	0.06[c]	0.06[c]	<0.01	0.01
20:4, *cis*-5, *cis*-8, *cis*-11, *cis*-14	2.10[a]	2.08[a]	2.04[ab]	2.00[b]	0.03	0.04
Others	18.65	18.76	18.51	18.72	0.30	0.49
Ratio						
16:0/*cis*-9 16:1	39.13	38.51	39.28	39.79	1.34	0.89
18:0/*cis*-9 18:1	5.51[b]	4.73[c]	6.09[a]	5.34[b]	0.07	0.01
trans-11 18:1/*cis*-9, *trans*-11 CLA	7.49[d]	10.07[b]	8.76[c]	12.20[a]	0.40	0.01

[1] Statistical probability of treatment differences. Values represent means for the last two milkings of the treatment period and means within row with different superscripts differ ($P < 0.05$).

Table 6
Composition of milk fat during abomasal infusion of partially hydrogenated vegetable oil (PHVO) and sterculic oil (SO) in lactating dairy cows

Fatty Acid	Treatment				SEM	P^1
	Control	PHVO	SO	PHVO + SO		
Content (g/100 g fatty acids)						
4:0	5.67[a]	4.90[b]	5.46[a]	4.95[b]	0.17	0.02
6:0	2.40[ab]	2.24[b]	2.67[a]	2.21[b]	0.10	0.01
8:0	1.20[ab]	1.08[b]	1.30[a]	1.04[b]	0.06	0.01
10:0	2.45[a]	1.85[c]	2.37[a]	2.15[b]	0.04	0.01
12:0	2.56[a]	2.12[c]	2.35[b]	2.31[b]	0.03	0.01
14:0	9.56[a]	7.96[c]	9.74[a]	9.21[b]	0.10	0.01
14:1, *cis*-9	0.55[a]	0.50[a]	0.09[b]	0.11[b]	0.02	0.01
15:0	0.81[a]	0.72[c]	0.78[b]	0.73[c]	<0.01	0.01
16:0	26.26[a]	23.25[b]	27.24[a]	24.08[b]	0.29	0.01
16:1, *cis*-9	0.73[a]	0.75[a]	0.30[b]	0.26[b]	0.03	0.01
17:0	0.47[b]	0.43[c]	0.52[a]	0.47[b]	0.01	0.01
18:0	14.30[c]	13.62[c]	23.07[a]	20.15[b]	0.35	0.01
18:1, *trans*-6 to 8	0.39[d]	0.78[b]	0.49[c]	0.89[a]	0.03	0.01
18:1, *trans*-9	0.28[c]	2.12[a]	0.29[c]	1.96[b]	0.03	0.01
18:1, *trans*-10	0.62[b]	2.09[a]	0.69[b]	2.16[a]	0.03	0.01
18:1, *trans*-11	2.18[d]	3.03[b]	2.57[c]	3.81[a]	0.05	0.01
18:1, *trans*-12	0.63[d]	1.37[b]	0.74[c]	1.51[a]	0.02	0.01
18:1, *cis*-9	20.66[a]	21.22[a]	11.21[c]	12.14[b]	0.29	0.01
18:2, *cis*-9, *cis*-12	4.04[b]	4.32[a]	4.02[b]	4.37[a]	0.08	0.01
18:3, *cis*-9, *cis*-12, *cis*-15	0.50	0.49	0.46	0.49	0.01	0.09
cis-9, *trans*-11 CLA	0.65[b]	0.76[a]	0.23[d]	0.30[c]	0.02	0.01
Others	1.79	2.30	2.18	1.86	0.06	0.01
Ratio						
14:0/*cis*-9 14:1	16.82[c]	16.26[c]	111.65[a]	85.82[b]	4.76	0.01
16:0/*cis*-9 16:1	65.32[b]	31.55[b]	118.84[a]	115.20[a]	10.74	0.01
18:0/*cis*-9 18:1	0.73[c]	0.64[c]	2.06[a]	1.66[b]	0.03	0.01
trans-11 18:1/*cis*-9, *trans*-11 CLA	3.59[c]	3.99[c]	11.63[b]	13.08[a]	0.39	0.01

[1] Statistical probability of treatment differences. Values represent means for the last two milkings of the treatment period and means within row with different superscripts differ ($P < 0.05$).

are subtle differences in fatty acids between treatments which are graphically illustrated in Figures 1 and 2. With infusion of PHVO+SO, *trans*-11 C18:1 was greater than observed for the PHVO treatment. By inhibiting desaturase, the conversion of exogenous *trans*-11 C18:1 fatty acids to *cis*-9, *trans*-11 CLA was markedly reduced, resulting in the observed increase over the PHVO treatment. However, the inhibition by sterculic oil was incomplete as indicated by the milk fat content of *cis*-9, *trans*-11 CLA which was greater during the PHVO+SO treatment than observed for the SO treatment (Fig. 1). Thus, the supply of *trans*-11 C18:1 and incomplete inhibition of Δ^9-desaturase resulted in greater *cis*-9, *trans*-11 CLA with the PHVO+SO treatment.

Δ^9-Desaturase adds a double bond across carbons 9 and 10. *trans*-8, *trans*-9, and *trans*-10 C18:1 fatty acids all have a pre-existing double bond at the site where Δ^9-desaturase would act. However, other measured *trans* fatty acids do not have this complication and consistent with a desaturation of these fatty acids, the SO treatment increased the milk fat content of *trans*-12 C18:1 and the coeluting *trans*-6–8 C18:1 in a manner similar to *trans*-11 C18:1 (Fig. 1 and *2*). In contrast, the milk fat content of *trans*-9 C18:1 and *trans*-10 C18:1 were identical for PHVO and PHVO+SO treatments consistent with the inability of these fatty acids to serve as substrates for Δ^9-desaturase.

4. Discussion

There is interest in increasing the concentration of *cis*-9, *trans*-11 CLA in milk fat. This stems from recent findings that *cis*-9, *trans*-11 CLA is the predominant isomer found in milk fat from dairy cows and it has anticarcinogenic effects in biomedical studies with animal models [2,3]. Diet has a major effect on milk fat concentration of *cis*-9, *trans*-11 CLA and dietary conditions which increase milk fat content have been described [19]. The *cis*-9, *trans*-11 CLA in milk fat has been assumed to represent *cis*-9, *trans*-11 CLA produced in the rumen by biohydrogenation of dietary polyunsaturated fatty acids [4,20]. While ruminal production of *cis*-9, *trans*-11 CLA contributes to milk fat CLA, we demonstrated that endogenous synthesis of *cis*-9, *trans*-11 CLA also makes a significant contribution [5]. Endogenous synthesis of *cis*-9, *trans*-11 CLA involves the enzyme Δ^9-desaturase and the substrate is *trans*-11 C18:1, another intermediate formed in the ruminal biohydrogenation of polyunsaturated C18-fatty acids. In order to quantify the

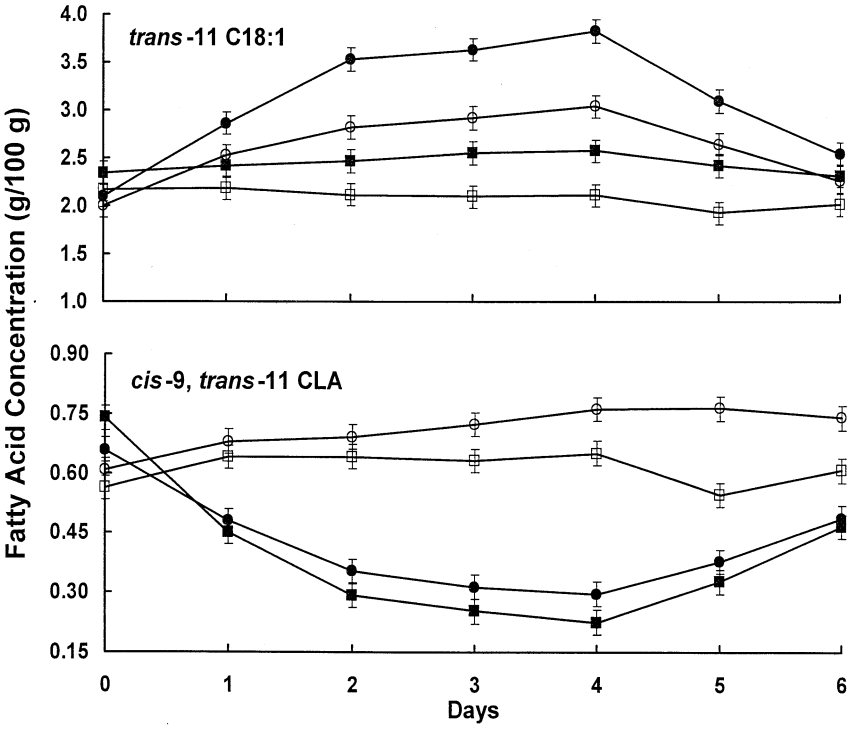

Fig. 1. Temporal pattern of *trans*-11 C18:1 (upper panel) and *cis*-9, *trans*-11 CLA (lower panel) in milk fat of lactating dairy cows receiving abomasal infusion of partially hydrogenated vegetable oil (250 g/day), sterculic oil (8.8 g/day), or both. Treatment commenced on day 1 and lasted through day 4. Values represent the mean of 4 cows with 2 milkings per day and bars on each data point indicate SE. Open squares = control, closed squares = sterculic oil, open circles = partially hydrogenated vegetable oil, and closed circles = partially hydrogenated vegetable oil plus sterculic oil.

relative contribution of endogenous synthesis, the present study supplied *trans*-11 C18:1 as exogenous substrate for desaturation to *cis*-9, *trans*-11 CLA as well as cyclopropene fatty acids to inhibit Δ^9-desaturase.

PHVO served as the source of *trans*-11 C18:1 and SO provided the cyclopropene fatty acids. Abomasal infusion of PHVO increased the milk fat content of CLA and *trans*-11 C18:1 by 17% and 39%, respectively. Likewise, inhibition of Δ^9-desaturase with SO dramatically reduced milk fat CLA. The reduction was 65% for SO versus control treatments and 61% for the comparison of PHVO+SO and PHVO treatments. Therefore, the results indicate endogenous synthesis is of major importance in supplying the CLA found in milk fat. Reductions observed with SO represent a minimum estimate of endogenous synthesis because complete inhibition of Δ^9-desaturase was not achieved. This is indicated by the PHVO+SO treatment that yielded a milk fat content of *cis*-9, *trans*-11 CLA significantly greater than the SO treatment. Incomplete inhibition is also illustrated by the presence of *cis*-9 C14:1 in milk fat for the SO and PHVO+SO treatments. Myristic acid (C14:0) is predominantly synthesized *de novo* by the mammary gland and *cis*-9 C14:1 originates from desaturation of C14:0 by Δ^9-desaturase located in mammary epithelial cells [21]. Consequently, the magnitude of the SO-induced reduction in C14:1 provides an estimate of the inhibition of Δ^9-desaturase. When this correction factor was applied, estimated endogenous synthesis was 78% of the total *cis*-9, *trans*-11 CLA in milk fat. Thus, endogenous synthesis was the major source of *cis*-9, *trans*-11 CLA in milk fat of lactating cows. In a previous experiment [5], endogenous synthesis was estimated to account for 64% of the total *cis*-9, *trans*-11 CLA in milk fat.

Cyclopropene fatty acids are found in the oils of many plant seeds including seeds from tropical trees, but also common flowers [22]. In the case of animal feeds, cottonseed oil contains measurable quantities of cyclopropene fatty acids and they can negatively impact animal performance due to inhibition of Δ^9-desaturase [22]. For example, in chickens, cyclopropene fatty acids inhibit Δ^9-desaturase and yolks become hard following refrigeration due to increased saturation of the yolk fatty acids [22]. However, ruminants do not typically experience negative effects since cyclopropene fatty acids represent a minor portion of the total diet and ruminal biohydrogenation inactivates them [23]. The sterculic oil used in the present study contained sterculic acid and malvalic acid (Table 2), both of which have a cyclopropene ring involving carbon 9. Jeffcoat and Pollard [9] demonstrated that fatty acids with a cyclopropene ring in this position are very potent inhibitors of Δ^9-desaturase. Others have also used cyclopropene fatty acids to inhibit Δ^9-desaturase in lactating ruminants at doses similar to the present study [5,23–25].

Milk fat contains several pairs of fatty acids that reflect a substrate-product relationship for Δ^9-desaturase. Four of these pairs are easily measured and ratios of these fatty acid

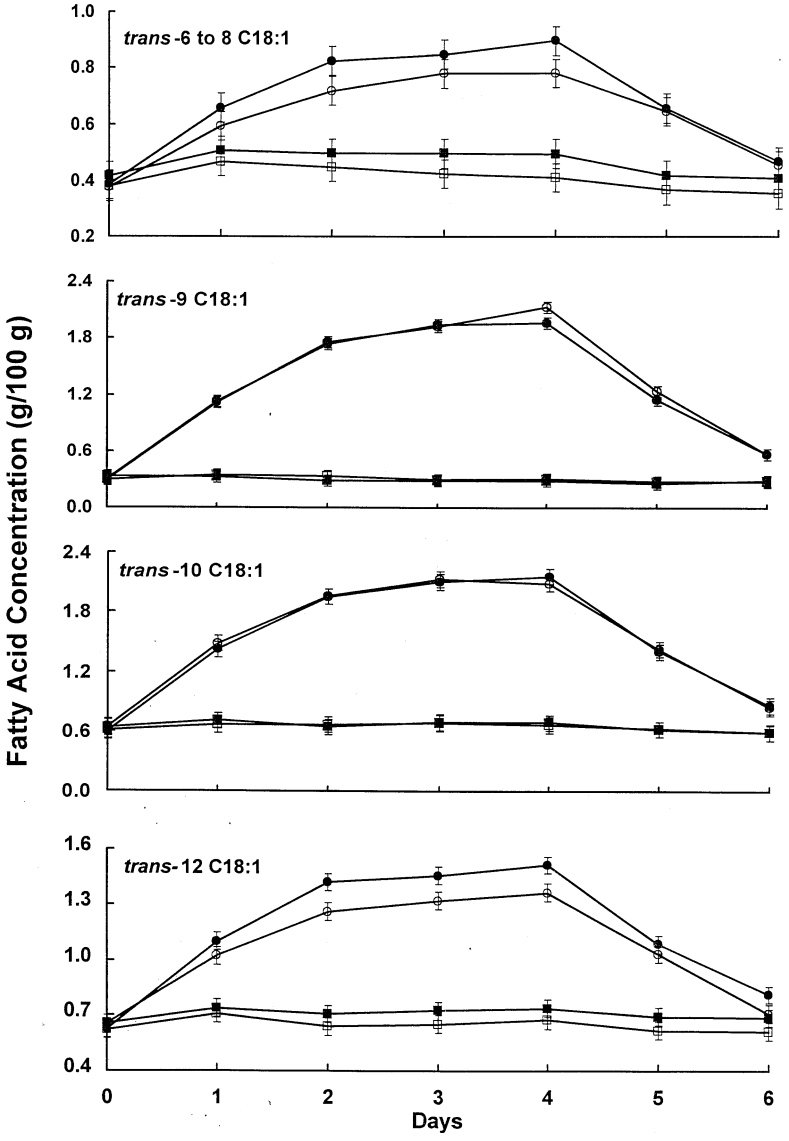

Fig. 2. Temporal pattern of *trans*-6–8 C18:1, *trans*-9 C18:1, *trans*-10 C18:1, and *trans*-12 C18:1 in milk fat of lactating dairy cows receiving abomasal infusion of partially hydrogenated vegetable oil (250 g/day), sterculic oil (8.8 g/day), or both. Treatment commenced on day 1 and lasted through day 4. Values represent the mean of 4 cows with 2 milkings per day and bars on each data point indicate SE. Open squares = control, closed squares = sterculic oil, open circles = partially hydrogenated vegetable oil, and closed circles = partially hydrogenated vegetable oil plus sterculic oil.

pairs were markedly altered when Δ^9-desaturase was inhibited with SO treatment (Table 6). Effects on the ratios of the Δ^9-desaturase fatty acid pairs were much smaller in plasma, although *cis*-9, *trans*-11 CLA and *cis*-9 C18:1 were reduced with SO treatment (Table 5). Thus, the mammary gland must be the major site of Δ^9-desaturase in lactating cows. A similar conclusion was reached by Bickerstaffe and Johnson [24] when they intravenously infused SO to a single goat. Studies comparing mRNA abundance [26] and activity [27–29] for Δ^9-desaturase have also demonstrated the mammary gland to be the major site for Δ^9-desaturase, although adipose tissue and intestine have detectable mRNA and/or enzyme activity [26,30,31]. Liver is a major site of Δ^9-desaturase in rodents [32] and this contrasts with ruminants. Relatively low abundance of Δ^9-desaturase mRNA has been observed in ruminant liver [26] and some studies have even failed to detect mRNA and enzyme activity in the liver [31,33].

The use of SO to inhibit Δ^9-desaturase also offers a unique opportunity to examine the role of this enzyme in the synthesis of milk fatty acids in general. Differences in milk fat content of *trans*-11 C18:1 between control versus SO treatments and PHVO versus PHVO+SO treatments reflect Δ^9-desaturase use of *trans*-11 C18:1 in the synthesis of *cis*-9, *trans*-11 CLA (Fig. 1). In contrast, milk fat content of *trans*-9 C18:1 and *trans*-10 C18:1 were not different for the same treatment comparisons because these fatty acids can not serve as substrates for Δ^9-desaturase. However, there were differences between control vs. SO and PHVO vs. PHVO+SO in milk fat content of *trans*-6–8 C18:1 and

trans-12 C18:1 indicating these fatty acids serve as substrates of Δ^9-desaturase. We previously demonstrated addition of a *cis*-9 bond to *trans*-12 C18:1 produces the *cis*-9, *trans*-12 C18:2 found in milk fat [5]. Differences in *trans*-6–8 C18:1 probably reflect use of *trans*-7 C18:1 to synthesize *trans*-7, *cis*-9 CLA, although methods used in the present study do not allow separation of this fatty acid. The presence of *trans*-7, *cis*-9 CLA in milk fat of ruminants has recently been reported by Yurawecz et al. [34] using more sophisticated analytical techniques.

Milk yield and milk protein were not altered by treatments, although milk fat yield was slightly reduced by SO and PHVO treatments. Bickerstaffe and Johnson [24] also observed a decrease in milk fat yield with SO. This may relate to the plasticity needs for milk fat triglycerides to be secreted, as the unsaturated fatty acids arising from Δ^9-desaturase would play an important role in establishing the physical characteristics of milk fat (see review by Chilliard et al. [35]). Others have also reported a decrease in milk fat yield when large doses of PHVO are infused (see review by Griinari and Bauman [4]). This apparently relates to the presence of specific fatty acids with a *trans*-10 double bond that inhibit milk fat synthesis, especially *de novo* synthesis.

Overall, results from the present study clearly demonstrate that endogenous synthesis is the major source of *cis*-9, *trans*-11 CLA in milk fat of lactating cows. The rumen is a source for a lesser portion of the *cis*-9, *trans*-11 CLA found in milk fat, but of greater importance is rumen production of *trans*-11 C18:1 which serves as the substrate for endogenous synthesis of CLA. Thus, the substantial variation which has been observed in milk fat content of CLA [19] is likely to be related to differences in ruminal production of *trans*-11 C18:1 as well as animal differences in Δ^9-desaturase activity. Interestingly, humans and other species are also capable of endogenous synthesis of CLA via Δ^9-desaturase with *trans*-11 C18:1 consumed in the diet serving as the substrate [3,6–8].

Acknowledgments

The contributions of Jodi Sangster, Lisa Perrin, Charity Sawyer, Bill English, and Dottie Ceurter for animal care and technical assistance are greatly appreciated. The assistance of Mike Rudan and David Barbano for preparation of emulsion solutions, and L.V. Ratnan and K.V. Raman for assistance in obtaining *Sterculia foetida* seeds is also gratefully acknowledged.

Supported in part by National Dairy Council (Rosemont, IL), Valio (Helsinki, Finland), Northeast Dairy Foods Research Center and the Cornell Agricultural Experiment Station.

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