Analysis of Variation in *cis*-9, *trans*-11 Conjugated Linoleic Acid (CLA) in Milk Fat of Dairy Cows¹

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ABSTRACT

Conjugated linoleic acid (CLA) is a fatty acid with numerous putative health benefits and is a natural component of ruminant-derived food products. An intermediate in rumen biohydrogenation is *cis*-9, *trans*-11 CLA, the major CLA isomer in milk fat. However, the major source of *cis*-9, *trans*-11 CLA in milk is endogenous synthesis by Δ^9 -desaturase conversion of *trans*-11 C_{18:1}, another rumen biohydrogenation intermediate. The desaturase indices serve as a proxy for Δ^9 -desaturase activity and are calculated from the ratios of fatty acid pairs that represent product/substrate for this enzyme. This study analyzed individual animal variation in milk fat content of *cis*-9, *trans*-11 CLA and in desaturase indices in milk fat. Thirty lactating Holstein cows were allocated to one of three treatment groups: one received a standard total mixed ration, one received a diet that produced an elevated milk fat content of CLA, and a third treatment group was alternated between these diets at 3-wk intervals over the 12-wk study. There was a two- to threefold variation among individuals on the same diet for both milk fat content of CLA and desaturase indices in milk fat. This hierarchy was maintained to a large extent over the 12-wk study even in the variable treatment group that alternated between the two diets. Within the variable diet treatment, some animals consistently had a substantial response in milk fat content of CLA to dietary shifts, whereas other cows had little or no response. We conclude that while diet is a major determinant of the CLA content in milk fat, individual animal differences also have a substantial effect. The variation among individuals includes differences related to both rumen biohydrogenation and Δ^9 -desaturase activity in the mammary gland.

(**Key words:** conjugated linoleic acid, milk fat, Δ^9 -desaturase, variation)

Abbreviation key: CLA = conjugated linoleic acid, ED = extruded full-fat soybean diet treatment, SD = standard diet treatment, VD_E = variable diet treatment when receiving the ED treatment diet, VD_S = variable diet treatment when receiving the SD treatment diet.

INTRODUCTION

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds. Biomedical studies with animal models have demonstrated a variety of beneficial health effects from CLA, including anticarcinogenic, antiatherogenic, antiobesity, immune system enhancement and antidiabetic (McGuire and McGuire, 2000; Whigham et al., 2000; Pariza et al., 2001). The various health effects of CLA may relate to specific CLA isomers, and this is an active area of research. However, it has been established that the *cis*-9, *trans*-11 CLA is anticarcinogenic when included in the diet as a natural component of food (Ip et al., 1999), and *cis*-9, *trans*-11 C_{18:2} is the major CLA isomer in dairy products (Parodi, 1997). The major source of *cis*-9, *trans*-11 CLA in milk fat is endogenous synthesis by Δ^9 -desaturase with the precursor being *trans*-11 C_{18:1}, an intermediate formed in rumen biohydrogenation of polyunsaturated fatty acids (Griinari et al., 2000; Corl et al., 2001). CLA is also an intermediate in rumen biohydrogenation, and a portion is absorbed and provides the remainder of the CLA in milk fat (Bauman et al., 2000b). In addition to endogenous synthesis of *cis*-9, *trans*-11 CLA, Δ^9 -desaturase catalyzes the introduction of a *cis*-9 double bond in several other fatty acids found in milk fat. Ratios for the pairs of fatty acids that represent product/substrate relationships are a proxy for Δ^9 -desaturase activity (Bauman et al., 2001).

Factors affecting the milk fat content of CLA have been reviewed, and diet plays a major role (Griinari and Bauman, 1999; Bauman et al., 2001). However, previous investigations have observed substantial variation in milk fat content of CLA among individual cows fed the same diet (Jiang et al., 1996; Kelly et al., 1998a; 1998b; Lawless et al., 1998). An analysis of this varia-

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Table 1. Nutrient composition of experimental diets.

Composition	Treatment diets ¹	
	SD	ED
Ingredients, % of DM		
Corn silage	42.6	40.7
Alfalfa silage	25.5	23.1
High moisture corn	12.8	12.1
Soybean meal	6.4	5.5
Soy plus	6.9	0
ESB ²	0	13.4
Dicalcium phosphate	0.2	0.2
Limestone	0.2	0.2
Mineral and vitamin mix	0.1	0.1
Sodium bicarbonate	0.6	0.6
Brome hay	4.7	4.1
Chemical, % of DM		
CP	17.7	17.8
NDF	36.7	35.0
ADF	20.2	21.4
DM, % of diet	44.5	46.0
NE _L , Mcal/kg	1.6	1.6

¹SD = Standard TMR diet and ED = diet containing extruded full-fat soybeans.

²ESB = Extruded full-fat soybeans.

tion was the focus of the present study. One objective was to examine the hierarchy of individual cows over time in the milk fat content of CLA. Given the importance of endogenous synthesis of CLA, a second objective was to examine the individual hierarchy in the milk fat ratios for fatty acid pairs related to Δ^9 -desaturase activity. We examined these hierarchies over a 12-wk period for three treatment groups, one fed a diet that resulted in a typical milk fat content of CLA, one fed a diet that resulted in an elevated milk fat content of CLA, and a third treatment group that was switched back and forth between the two diets at 3-wk intervals.

MATERIALS AND METHODS

Animals and Treatments

All procedures involving dairy cows were approved by the Cornell University Institutional Animal Care and Use Committee. Thirty lactating Holstein cows were blocked according to parity (9 multiparous, 21 primiparous) and DIM (96.4 ± 2.3 ; mean $\pm$ SE) and randomly allocated to one of three treatments. Cows were housed in group pens by treatment at the Cornell University Teaching and Research Center (Harford, NY).

Treatments involved feeding diets that were formulated to give differences in the milk fat content of CLA (Table 1). The first treatment group received a traditional corn-based TMR for the duration of the 12-wk study; this diet would produce a standard milk fat content of CLA (**SD** treatment). The second group received

a diet that included extruded full-fat soybeans for the 12-wk period; this diet would result in an elevated milk fat content of CLA (**ED** treatment). The third group (variable diet; **VD** treatment) was alternated between the two diets at 3-wk intervals (four periods) so that during periods 1 and 3 they were fed the standard diet (**VD_S**) and during periods 2 and 4, the extruded full-fat soybean diet (**VD_E**).

Diets were formulated using the Cornell Net Carbohydrate and Protein System (Fox et al., 1992) to meet or exceed nutrient requirements (NRC, 1989). Cows were fed ad libitum, with fresh feed given once daily, and they had free access to water. For analyses, feed samples were taken once per week directly after feeding and dried at 50°C for 72 h for DM determination. Samples were composited at 4-wk intervals for analysis of composition by wet chemistry methods (Dairy One Cooperative, Inc., Ithaca, NY).

Cows were milked 3 times per day. Milk yield was recorded daily and duplicate samples collected from each milking 1 d/wk. One set was analyzed for fat and protein by infrared analysis (Dairy One Cooperative, Inc.). The second set of milk samples was combined based on milk yield to obtain an individual cow's weekly composite sample that was analyzed for fatty acid composition.

Fatty Acid Analysis

Composited milk samples were centrifuged at 17,800 $\times g$ for 30 min at 8°C, and 300 to 400 mg of fat cake was removed for extraction and methylation. Lipid extraction was performed by the method of Hara and Radin (1978) and methyl esters of the fatty acids were prepared by transesterification with sodium methoxide according to the method of Christie (1982) as detailed by Chouinard et al. (1999). Fatty acid methyl esters were quantified by gas chromatography using a SP-2560 capillary column (100 m $\times$ 0.25 mm i.d. with 0.2 μ m film thickness; Supelco Inc., Bellefonte, PA). The analysis 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 flow rate for hydrogen carrier gas 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.

Each peak was identified and quantified using pure methyl ester samples (Nu Chek Prep, Elysian, MN). A butter reference standard (CRM 164; Commission of the European Communities, Community Bureau of Ref-

Table 2. Effect of treatment on milk production.¹

Variable	Treatments ²				<i>P</i> ³
	SD	ED	VD _S	VD _E	
Milk yield, kg/d	35.5 ± 1.1 ^{ab}	37.3 ± 1.1 ^a	32.9 ± 1.2 ^b	32.7 ± 1.2 ^b	0.03
Milk protein					
%	2.75 ± 0.05	2.79 ± 0.05	2.84 ± 0.05	2.83 ± 0.06	0.66
kg/d	0.97 ± 0.03 ^{ab}	1.05 ± 0.03 ^a	0.91 ± 0.03 ^b	0.87 ± 0.04 ^b	0.01
Milk fat					
%	3.33 ± 0.11	3.09 ± 0.11	3.43 ± 0.11	3.49 ± 0.13	0.13
kg/d	1.20 ± 0.05	1.12 ± 0.05	1.14 ± 0.05	1.09 ± 0.06	0.21

¹Each treatment had 10 cows and values are mean ± SE for 12 wk (SD and ED) or 6 wk (VD_S and VD_E).

²SD = Standard diet treatment, ED = extruded full-fat soybean diet treatment, VD_S = variable diet treatment on the standard diet, and VD_E = variable diet treatment on the extruded full-fat soybean diet.

³When a treatment effect was significant (*P* < 0.05), differences among treatments were compared by *t*-test as indicated by superscripts within a row (a, b; *P* < 0.05).

erence, Brussels, Belgium) was used to determine recoveries and correction factors for individual fatty acids. The butter reference standard was also used at regular intervals throughout the analysis as an aid in quality control.

Statistical Analyses

Performance and fatty acid data were analyzed as an unbalanced repeated measures design using the PROC MIXED procedure of SAS (2000) according to the model:

$$Y_{ijk} = \mu + D_i + P_j + C_k + \varepsilon_{ijk},$$

where *Y* is the response variable, μ is the overall mean, *D_i* is the dietary treatment, *P_j* is the period effect, *C_k* is the cow effect (treated as a random factor), and ε_{ijk} is the residual error term. The effect of treatment was analyzed as a fixed effect in a mixed ANOVA model, with cow effects treated as a random factor. If the overall *F*-test of treatment was significant (*P* < 0.05), then a Fisher's *t*-test was performed to discern differences among the treatments ("lsmeans/pdiff" command in SAS). A term was initially included in the model for period × diet interaction, but it was not significant and was dropped from the final model. The PROC REG function of SAS was used to compute the regression equation and correlation coefficient between *trans*-11 C_{18:1} and *cis*-9, *trans*-11 CLA. Correlation of ratios for the fatty acid pairs that are proxies for Δ^9 -desaturase was determined using the PROC CORR procedure of SAS.

RESULTS

Cows were grouped by treatment (*n* = 10 cows/treatment) and DMI (kg/d) averaged 185 ± 3, 181 ± 3, 182 ± 4, and 191 ± 4 (mean ± SE) for SD, ED, VD_S and VD_E, respectively. Milk production and milk composition are reported in Table 2. Milk fat percent and milk fat yield

were similar among treatment groups. Cows on the ED treatment exhibited higher milk production and a slightly greater milk protein yield, although milk protein percent was not affected by diet.

Fatty acid composition of the milk fat is presented in Table 3. Cows consuming the extruded full-fat soybean diet exhibited a greater proportion of long-chain (≥18 carbon) fatty acids with major increases occurring for stearic acid, oleic acid and linoleic acid. In contrast, cows fed the standard corn-based TMR diet produced milk fat with a greater proportion of short and medium chain fatty acids and an increased content of palmitic acid.

Milk fat content of *cis*-9, *trans*-11 CLA was successfully elevated by the ED treatment (Table 3), and the elevated level was maintained for the duration of the 12-wk study (Figure 1). Alternating the diet for the VD treatment group resulted in rapid shifts in the CLA content in milk fat that matched the *cis*-9, *trans*-11 CLA content of the SD and ED treatment groups in corresponding periods (Figure 1).

Figure 2 presents the *cis*-9, *trans*-11 CLA concentrations in milk fat for individual cows on the study averaged over each 3-wk period. There was a two- to three-fold range in CLA concentration among individuals regardless of diet. The VD treatment group that was switched between the two diets also exhibited the same two- to threefold range. Individuals in all three treatments were quite consistent in their position in the hierarchy of *cis*-9, *trans*-11 CLA concentration in milk fat across all four periods.

Trans-11 C_{18:1} and *cis*-9, *trans*-11 CLA represent a precursor-product relationship for endogenous synthesis via Δ^9 -desaturase, and the correlation between them was 0.61 across all cows and treatments (Figure 3). The ratio of *cis*-9, *trans*-11 CLA to *trans*-11 18:1 has been referred to as the desaturase index (Choi et al., 2000), and there are three additional fatty acid pairs in milk fat that also reflect this relationship for Δ^9 -desaturase,

Table 3. Effect of treatment on fatty acid composition of milk fat.¹

Fatty acid	Treatments ²				P ³
	SD	ED	VD _S	VD _E	
	g/100 g of fatty acids				
C _{4:0}	5.96 ± 0.12	5.66 ± 0.12	5.64 ± 0.12	5.73 ± 0.15	0.18
C _{6:0}	2.77 ± 0.08 ^a	2.48 ± 0.07 ^b	2.80 ± 0.08 ^a	2.70 ± 0.09 ^a	0.001
C _{8:0}	1.29 ± 0.04 ^{ac}	1.04 ± 0.04 ^b	1.31 ± 0.04 ^a	1.19 ± 0.05 ^c	0.001
C _{10:0}	2.52 ± 0.12 ^{ab}	2.21 ± 0.12 ^a	2.74 ± 0.12 ^b	2.31 ± 0.13 ^a	0.001
C _{12:0}	2.85 ± 0.12 ^a	2.48 ± 0.12 ^b	2.99 ± 0.12 ^a	2.45 ± 0.12 ^b	0.001
C _{14:0}	10.51 ± 0.23 ^a	9.05 ± 0.23 ^b	10.48 ± 0.24 ^a	9.07 ± 0.24 ^b	0.001
c9 C _{14:1}	0.94 ± 0.05 ^a	0.76 ± 0.05 ^{bc}	0.87 ± 0.06 ^{ac}	0.75 ± 0.04 ^b	0.001
C _{16:0}	30.81 ± 0.83 ^a	24.54 ± 0.83 ^b	28.60 ± 0.89 ^a	23.96 ± 0.83 ^b	0.001
c9 C _{16:1}	1.60 ± 0.07 ^a	1.34 ± 0.07 ^b	1.57 ± 0.08 ^a	1.26 ± 0.08 ^b	0.001
C _{18:0}	9.93 ± 0.41 ^a	11.82 ± 0.41 ^{bc}	10.93 ± 0.41 ^{ac}	12.44 ± 0.41 ^a	0.001
t6 to t8 C _{18:1}	0.32 ± 0.02 ^a	0.54 ± 0.02 ^b	0.36 ± 0.03 ^a	0.52 ± 0.03 ^b	0.001
t9 C _{18:1}	0.25 ± 0.02 ^a	0.44 ± 0.02 ^b	0.29 ± 0.02 ^a	0.45 ± 0.02 ^b	0.001
t10 C _{18:1}	0.62 ± 0.13 ^a	0.96 ± 0.13 ^b	0.71 ± 0.13 ^a	1.08 ± 0.14 ^b	0.001
t11 C _{18:1}	1.03 ± 0.10 ^a	1.75 ± 0.10 ^b	1.12 ± 0.10 ^a	2.18 ± 0.11 ^c	0.001
c9 C _{18:1}	21.39 ± 0.76 ^a	25.11 ± 0.76 ^b	22.69 ± 0.76 ^a	25.50 ± 0.80 ^b	0.001
c9, c12 C _{18:2}	2.50 ± 0.12 ^a	4.27 ± 0.12 ^b	2.56 ± 0.12 ^a	3.20 ± 0.14 ^c	0.001
c9, c12, c15 C _{18:3}	0.49 ± 0.02 ^a	0.63 ± 0.01 ^b	0.45 ± 0.02 ^a	0.56 ± 0.02 ^c	0.001
c9, t11 CLA	0.51 ± 0.03 ^a	0.80 ± 0.03 ^b	0.52 ± 0.03 ^a	0.87 ± 0.04 ^b	0.001
others	3.41 ± 0.09 ^a	3.94 ± 0.09 ^b	3.61 ± 0.13 ^a	3.97 ± 0.13 ^b	0.001

¹Each treatment had 10 cows and values are mean ± SE for 12 wk (SD and ED) or 6 wk (VD_S and VD_E).

²SD = Standard diet treatment, ED = extruded full-fat soybean diet treatment, VD_S = variable diet treatment on the standard diet and VD_E = Variable diet treatment on the extruded full-fat soybean diet.

³When a treatment effect was significant ($P < 0.05$), differences among treatments were compared by *t*-test as indicated by superscripts within a row (a, b, c; $P < 0.05$).

cis-9 C_{14:1}/C_{14:0}, *cis*-9 C_{16:1}/C_{16:0}, and *cis*-9 C_{18:1}/C_{18:0}. The effect of treatment was compared for all four desaturase indices (Table 4). The ratio of *cis*-9, *trans*-11 CLA/*trans*-11 C_{18:1} was lower for cows fed the diet containing extruded full-fat soybeans, but there were no treatment differences for the ratios of other fatty acid pairs.

Figure 1. Weekly treatment averages in milk fat content of *cis*-9, *trans*-11 conjugated linoleic acid (CLA). Treatment groups ($n = 10$ cows per treatment) included cows receiving a standard TMR diet (SD treatment), a diet including extruded full-fat soybeans (ED treatment), and a variable diet group (VD treatment) that alternated between diets at 3-wk intervals so that they received the SD treatment diet during periods 1 and 3, and the ED treatment diet during periods 2 and 4. Pooled SE = 0.49 mg/g fatty acids.

The temporal pattern of the desaturase indices for individual cows was examined and values for *cis*-9 C_{14:1}/C_{14:0} and *cis*-9, *trans*-11 CLA/*trans*-11 C_{18:1} are presented in Figure 4. The range among individuals on the same treatment was about twofold. Individual cows on the SD and ED treatments showed a remarkable degree of consistency over the 12-wk experimental period in their position in the desaturase index hierarchy relative to other members of their treatment group. A consistent hierarchy was also maintained for the VD treatment group across dietary shifts (Figure 4). The range among individuals and the consistency over time were similar for the ratios of *cis*-9 C_{16:1}/C_{16:0} and *cis*-9 C_{18:1}/C_{18:0} (data not presented). There was also good agreement among the pairs of fatty acids related to Δ^9 -desaturase irrespective of diet. The correlation coefficients between ratios for the fatty acid pairs in the desaturase index ranged from 0.48 to 0.78 (Table 5) demonstrating the positive relationship among all the indices.

DISCUSSION

Conjugated linoleic acids have been shown to have a wide array of health benefits in studies with animal disease and cancer models (Whigham et al., 2000; Pariza et al., 2001). Dairy products accounted for approximately 60% of the CLA intake in US diets (Ritzenhaler

Figure 2. Milk fat content of *cis*-9, *trans*-11 CLA for individual cows on each treatment. Treatment groups included cows receiving a standard TMR diet (SD treatment), a diet including extruded full-fat soybeans (ED treatment), and a variable diet group (VD treatment) that alternated between diets at 3-wk intervals so that they received the SD treatment diet during periods 1 and 3, and the ED treatment diet during periods 2 and 4. Each symbol represents an individual cow ($n = 10$ cows per treatment) and each period value represents the mean of the three weekly values for that period. SE for individual period means averaged 0.035, 0.035, and 0.040 mg/g fatty acids for SD, ED and VD, respectively.

et al., 2001), and detailed analysis of milk fat has identified 19 different isomers of CLA (Sehat et al., 1998). However, the predominant CLA isomer is *cis*-9, *trans*-11, and it generally accounts for 75 to 90% of the total CLA present in milk fat (Sehat et al., 1998; Bauman

Figure 3. Regression of the milk fat content of *cis*-9, *trans*-11 CLA on *trans*-11 C_{18:1} for all cows ($n = 30$) over the 12-wk study. Each point represents a weekly value for an individual cow. $P < 0.001$.

et. al., 2000a). Diet of the cow has a major influence on the milk fat content of CLA, and these effects have been recently summarized (Bauman et al., 2000b, 2001). Polyunsaturated lipids are one important component, and in the present study extruded full-fat soybeans were fed as a slow-release source of linoleic acid. Across treatment groups we observed an increase in the milk fat content of *cis*-9, *trans*-11 CLA when cows consumed the diet containing extruded full-fat soybeans compared with cows fed the standard corn-based TMR (8.3 vs. 5.0 mg of CLA/g of fatty acids; $P < 0.0001$). Others have reported increases in milk fat content of CLA of similar or greater magnitude when extruded full-fat soybeans were fed (Lawless et al., 1998; Dhiman et. al., 1999; Solomon et al., 2000; Chouinard et al., 2001).

One objective was to examine the variation in milk fat content of CLA in individual cows over time and across different dietary treatments. Data demonstrated that average *cis*-9, *trans*-11 CLA content of milk fat for the ED and SD treatments was relatively constant over the 12-wk experimental period. The VD treatment group had a milk fat content of CLA similar to the SD and ED treatments during each period that they were receiving the same respective diet (Figure 1). On an individual cow basis, we observed a two- to threefold range in the milk fat content of CLA for each of the treatment groups (Figure 2). Previous studies involving diets ranging from TMR to pasture reported a similar two- to threefold range in milk fat content of CLA for individual cows (Jiang et al., 1996; Kelly et al., 1998a, 1998b; Lawless et. al., 1998; Solomon et al., 2000; White et al., 2001).

White et al. (2001) recently reported that individual cows maintained a relatively constant milk fat content of CLA over a 4-wk period. In the present study, comparison of the temporal pattern of milk fat content of *cis*-

Table 4. Effect of diet on milk fatty acid pairs in the desaturase index.

Fatty acid pair	Treatments ^{1,2}		SEM	P
	SD + VD _S	ED + VD _E		
c9, t11CLA/t11 C _{18:1}	0.52 ^a	0.44 ^b	0.02	0.01
c9 C _{14:1} /C _{14:0}	0.088	0.084	0.003	0.11
c9 C _{16:1} /C _{16:0}	0.054	0.053	0.001	0.38
c9 C _{18:1} /C _{18:0}	2.19	2.11	0.07	0.15

¹SD = Standard diet treatment, VD_S = variable diet treatment on the standard diet, ED = extruded full-fat soybean diet and VD_E = variable diet treatment on the extruded full-fat soybean diet.

²Treatments groups have been combined by diet and represent mean of all cows for all weeks on a given diet (12 wk for SD and 6 wk for VD_S; 12 wk for ED and 6 wk for VD_E).

9, *trans*-11 CLA over the 12-wk period indicated that the individual hierarchy in CLA levels was maintained to a substantial extent for both SD and ED treatments (Figure 2). Lawless et al. (1999) previously compared CLA content in milk samples obtained from individual cows at approximately 75 and 120 d postpartum; four breeds were compared, and correlations of 0.17 to 0.56 for the CLA content of the two samples were observed. Particularly revealing in the present study was the VD treatment group in which the hierarchy was maintained even as cows were switched back and forth between the two diets that resulted in very different milk fat concentrations of CLA (Figure 2). Interestingly, some animals in the VD treatment consistently had a substantial response in milk fat *cis*-9, *trans*-11 CLA to dietary shifts, whereas other cows had little or no response. This phenomenon was also observed by Kelly et al. (1998b) when animals were shifted from a corn-based TMR to a diet of only pasture. In the present study, cows were similar in milk production and stage of lactation, but they were group fed, so that the effect of intake on the variation in CLA content among individuals cannot be evaluated. Studies have observed that substantial underfeeding caused an increase (Jiang et al., 1996) or a decrease (Stanton et al., 1997) in group averages for CLA content of milk fat. However, variation among individuals was not considered, and we know of no study that has specifically examined the contribution of intake to individual variation in CLA content of milk fat. Based on the consistency of literature values in CLA content of milk fat across studies (see review by Bauman et al., 2001), it seems likely that subtle differences in feed intake among well-fed cows will be of little or no importance in explaining variation among individuals.

The *cis*-9, *trans*-11 CLA in milk fat originates from the incomplete biohydrogenation of polyunsaturated fatty acids (Griinari and Bauman, 1999). The majority of milk fat CLA is synthesized endogenously via Δ^9 -desaturase from *trans*-11 C_{18:1}, an intermediate in the rumen biohydrogenation of linoleic and linolenic acids

(Griinari et al., 2000; Corl et al., 2001). The remainder of the CLA in milk fat arises directly from CLA absorbed from the digestive tract after being produced in the rumen as an intermediate in linoleic acid biohydrogenation. Thus, milk fat content of *cis*-9, *trans*-11 CLA is dependent on rumen production of *trans*-11 C_{18:1} and CLA, as well as tissue activity of Δ^9 -desaturase (Bauman et al., 2001).

In the present study, the correlation coefficient was 0.61 between *cis*-9, *trans*-11 CLA and *trans*-11 C_{18:1} across all animals and diets ($P < 0.01$). The ratio of *cis*-9, *trans*-11 CLA to *trans*-11 C_{18:1} was affected by treatment, averaging 0.52 and 0.44 for the standard diet and the extruded full-fat soybean diet, respectively (Table 4). Other studies have reported milk fat ratios of 0.25 to 0.46 for *cis*-9, *trans*-11 CLA/*trans*-11 C_{18:1} under various dietary conditions (Jiang et al., 1996; Jahreis et al., 1997; Lawless et al., 1998; Griinari and Bauman, 1999). The lower ratio of *cis*-9, *trans*-11 CLA to *trans*-11 C_{18:1} on the extruded full-fat soybean diet where both substrate and product are present in milk fat at elevated concentrations (up to threefold), suggests saturation of the Δ^9 -desaturase system or a change in enzyme kinetics under these specific conditions. However, the desaturase index was not different for other fatty acid pairs in the desaturase index (Table 4). Another indication that the desaturase system is not likely to be limited by elevated concentrations of substrate or product is the investigation by Bauman et al. (2000a), which represented a unique situation wherein cows were fed and selected to produce CLA-enhanced milk fat; an increase was observed in both milk fat content of CLA (40.7 vs. 5.3 mg/g of fatty acids) and the milk fat ratio of *cis*-9, *trans*-11 CLA/*trans*-11 C_{18:1} (0.51 vs. 0.33). The second objective in the present study was to examine variation in the milk fat ratio of fatty acid pairs related to Δ^9 -desaturase. This enzyme is important in the maintenance of the fluidity of cellular membranes and milk fat (Parodi, 1982; Chilliard et al., 2000). There are four fatty acid pairs that represent a product/substrate relationship for Δ^9 -desaturase, and milk fat ra-

Figure 4. Desaturase index values for milk fat ratios of $cis-9\ C_{14:1}/C_{14:0}$ and $cis-9, trans-11\ CLA/trans-11\ C_{18:1}$. Treatment groups included cows receiving a standard TMR diet (SD treatment), a diet including extruded full-fat soybeans (ED treatment), and a variable diet group (VD treatment) that alternated between diets at 3-wk intervals so that they received the SD treatment diet during periods 1 and 3, and the ED treatment diet during periods 2 and 4. Each symbol represents an individual cow ($n = 10$ cows per treatment) and each period value represents the average of the three weekly values for that period. SE for individual period means for the C_{14} desaturase index values averaged 0.0051, 0.0051 and 0.0054 for SD, ED, and VD, respectively; for the CLA desaturase index values, SE averaged 0.034, 0.034 and 0.036 for SD, ED, and VD, respectively.

tios for these pairs of fatty acids represent a proxy for Δ^9 -desaturase activity (Bauman et al., 2001). In the present study, all four of the fatty acid pairs were positively associated among individuals across all diets, with the correlation coefficients ranging from 0.48 to 0.78 (Table 5). In examining the individual hierarchy related to endogenous synthesis of CLA, the ratio of myristoleic acid to myristic acid ($cis-9\ C_{14:1}/C_{14:0}$) may

represent the best proxy for Δ^9 -desaturase activity in the mammary gland. Myristic acid originates almost exclusively via de novo synthesis within the mammary gland, and, as a consequence, all of the myristoleic acid present in milk fat would also be synthesized in the mammary gland by Δ^9 -desaturase. In contrast, fatty acid absorption from the digestive tract and mobilization of body fat reserves can account for a large and

Table 5. Correlation coefficients between desaturase index values for the pairs of fatty acids representing product/substrate for Δ^9 -desaturase.¹

Fatty acid pair	c9, t11 CLA/t11 C _{18:1}	c9 C _{14:1} /C _{14:0}	c9 C _{16:1} /C _{16:0}	c9 C _{18:1} /C _{18:0}
c9, t11 CLA/t11 C _{18:1}	1	0.65	0.53	0.78
c9 C _{14:1} /C _{14:0}		1	0.48	0.66
c9 C _{16:1} /C _{16:0}			1	0.66
c9 C _{18:1} /C _{18:0}			1	1

¹Twelve-week averages for each cow were used in correlation analysis (n = 30 cows). All correlations were significant ($P < 0.01$).

variable proportion of palmitoleic, palmitic, oleic, stearic, and vaccenic acids as well as CLA, making these respective ratios less indicative of actual mammary activity of Δ^9 -desaturase.

This is the first study to extensively characterize individual variation in fatty acid pairs that represent a proxy for Δ^9 -desaturase activity. A range of approximately twofold in individual values was observed for all treatments for the ratios of *cis*-9 C_{14:1}/C_{14:0} and *cis*-9, *trans*-11 CLA/*trans*-11 C_{18:1} (Figure 4). A similar range in individual variation was observed for *cis*-9 C_{16:1}/C_{16:0} and *cis*-9 C_{18:1}/C_{18:0} (data not presented). There was also a relatively consistent hierarchy in the desaturase index ratios over time for the ED and SD treatments (Figure 4). Even more illuminating was the VD treatment in which the individual hierarchy was maintained across time (Figure 4), even when cows were repeatedly switched between diets that caused the milk fat concentration of both *cis*-9, *trans*-11 CLA and *trans*-11 C_{18:1} to vary approximately twofold (Table 3). The consistency of the individual hierarchy in desaturase index ratios suggests that a portion of the variation in milk fat content of CLA when cows are fed the same diet is related to individual differences in Δ^9 -desaturase activity. Presumably this would be represented by differences in the regulation of Δ^9 -desaturase gene expression, differences in primary or tertiary structure of the enzyme due to gene polymorphisms or posttranslational modifications or differences in downstream factors that would affect interaction between enzyme and substrate (e.g., phosphorylation). This is an obvious area for future research.

CONCLUSIONS

In the present study, substantial variation in milk fat CLA content between animals and across diets was observed, similar to previous studies. We also demonstrated that individual animals were quite consistent over time; the hierarchy of individual animals was maintained even when cows were switched between diets that give substantial differences in milk fat content of CLA. This suggests that rumen production of

trans-11 C_{18:1} and CLA varies among individuals on the same diet, and this would represent the basis for a portion of the individual variation in milk fat content of CLA. Δ^9 -desaturase is a key enzyme in the endogenous synthesis of CLA, and the milk fat ratios of fatty acid pairs that represent proxies for this enzyme also varied substantially even between animals on the same diet. This hierarchy among individuals was also relatively constant over the 12-wk experiment, even when cows were alternated between dietary treatments. Thus, differences among individuals in Δ^9 -desaturase contribute to variation in milk fat content of CLA. Our results indicate that while diet is a major determinant of CLA in milk fat, some animals possess a greater potential to respond to a dietary treatment than others, and that these differences in response are consistent over time and across multiple dietary shifts.

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