Trans-Octadecenoic Acids and Milk Fat Depression in Lactating Dairy Cows[1]

J. M. GRIINARI,*,[2] D. A. DWYER,* M. A. MCGUIRE,*,[3] D. E. BAUMAN,*,[4] D. L. PALMQUIST,† and K.V.V. NURMELA‡

*Department of Animal Science, Cornell University, Ithaca, NY 14853
†Department of Animal Sciences, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster 44691
‡Valio Ltd., FIN-00039 Helsinki, Finland

ABSTRACT

We examined the role of *trans*-octadecenoic acids in milk fat depression when low fiber diets were fed. The study consisted of four experimental periods with a 2 × 2 factorial arrangement of treatments to test the effects of dietary fat (saturated vs. unsaturated) and rumen fermentation (high fiber diets vs. low fiber diets) on milk fat depression. Dietary fiber concentration and type of fat had significant effects on milk fat. Effects were most pronounced when unsaturated fat was added to the low fiber diet. When the low fiber diet plus unsaturated fat was fed, milk fat percentage and yield were decreased by 30 and 35%, respectively, compared with the percentage and yield when the high fiber diet plus saturated fat was fed. Alterations in rumen fermentation caused by differences in dietary fiber concentrations had little effect on the amount of *trans*-octadecenoic acids in milk fat, and the total amount did not correlate with changes in milk fat percentage. Further examination of the isomeric profile of *trans*-octadecenoic acid revealed substantial differences among the dietary treatments. Although the addition of unsaturated fat resulted in marked increases in the milk fat content of *trans*-11-octadecenoic acid, regardless of dietary fiber concentration, the low fiber diet plus unsaturated fat increased the content of *trans*-10-octadecenoic acid. This combination was also associated with a significant decrease in milk fat content and yield. When the low fiber diets were fed, circulating insulin concentrations were elevated, regardless of the type of fat supplement. However, marked milk fat depression occurred only when the low fiber diet was supplemented with unsaturated fat.

(**Key words**: *trans*-octadecenoic acids, insulin, milk fat depression, fat synthesis)

Abbreviation key: **HF** = high fiber, **LF** = low fiber, **MFD** = milk fat depression, **SFA** = saturated fatty acids, **UFA** = unsaturated fatty acids.

INTRODUCTION

The NRC report *Designing Foods* (28) emphasized the need for research on the improvement of both the nutritional value and the labeling of foods. Today, both of these policy recommendations have been followed, and consumers are much more aware of nutrition, particularly dietary fat. This awareness is clearly indicated by the shift toward increased consumption of lowfat products in the fluid milk market. Changes in government policy have also had an impact on the milk market, and the rapid decrease in butter support price since 1988 has decreased the value of milk fat by more than 50% (15).

When high concentrate diets are fed, the rate of milk fat synthesis can decrease by 50% or more (12, 43, 48). In addition, several other dietary manipulations, including dietary fats that are active in the rumen, forages with small particle size, lush pasture, and ionophores, result in varying degrees in decreased milk fat yield (48). The actual mechanisms involved in milk fat depression (**MFD**) have not been fully elucidated, but several theories have been proposed. Most of these theories involve changes in rumen fermentation or metabolism, which are postulated to result in a shortage of lipid precursors at the mammary gland (1, 12, 43, 47).

One possible mechanism of MFD involves the production of metabolites in the rumen that directly inhibit mammary synthesis of milk fat. Direct inhibi-

Received May 16, 1997.
Accepted December 22, 1997.
[1]Research supported in part by the Northeast Dairy Foods Research Center and Cornell University Agricultural Experiment Station.
[2]Recipient of a scholarship from the Academy of Finland. Current address: Valio Ltd., PO Box 30, FIN-00039 Helsinki, Finland.
[3]Current address: University of Idaho, Department of Animal and Veterinary Science, 217 Ag Science Bldg., Moscow 83844-2330.
[4]Address correspondence to Dale E. Bauman, Cornell University, 262 Morrison Hall, Ithaca, NY 14853-4801.

tion of milk fat synthesis by partially hydrogenated fatty acids (specifically *trans*-octadecenoic acids) was first proposed more than two decades ago (12, 31). *Trans*-octadecenoic acids are produced by rumen microbes as intermediates in the biohydrogenation of unsaturated fatty acids. *Trans*-11-octadecenoic acid is a major intermediate in the formation of stearic acid from linoleic and linolenic acids (20). The objective of the present study was to examine the hypothesis that two conditions are required for the production of *trans*-octadecenoic acid and subsequent MFD: the presence of rumen substrate in the form of dietary unsaturated fatty acids (**UFA**) and an altered rumen environment, which leads to incomplete biohydrogenation.

MATERIALS AND METHODS

All procedures involving cows were approved by the Cornell University Institutional Animal Care and Use Committee. Four multiparous rumen-fistulated cows averaging 162 ± 19 DIM ($\bar{X}$ ± SEM) were used in a 2 × 2 factorial arrangement of treatments applied to a 4 × 4 Latin square design. Treatments included forage to concentrate ratios of either 50:50 (high fiber; **HF**) or 20:80 (low fiber; **LF**) to alter the rumen environment. Treatments also included the addition of either corn oil as a source of UFA or a dry fat product (Energy Booster 100®; Milk Specialties Co., Dundee, IL), which consisted of mostly (85%) saturated fatty acids (**SFA**). The forage source was chopped alfalfa hay, and all other dietary ingredients were chosen among those with the lowest fat content. The grain source was a commercial degermed yellow corn product (J. R. Short Milling Co., Kankakee, IL) with a low fat content (0.7% crude fat) and a specified particle size range (65 to 85% of particles were >1.7 mm; 15 to 35% of particles were >2.36 mm). Solvent-extracted soybean meal had 2.7% crude fat based on chemical analysis (Northeast Forage Testing Laboratory, Ithaca, NY). The calculated lipid content of the basal diet without added fat was 1.7 and 1.1% for HF and LF diets, respectively.

The study was conducted in the Large Animal Research and Teaching Unit, and cows were fed a total mixed diet (HF + SFA) for ad libitum intake for 1 wk after they were brought to this unit. Cows were then restricted (individual cow basis) to that level of maximum energy intake each time the LF diets were fed during the following 2-wk experimental periods. This strategy was chosen to minimize potential rumen upsets caused by excess consumption of the LF diet. Cows receiving the HF diet were allowed ad libitum consumption.

The first two cows that were randomly assigned to the LF diet were gradually adapted to the diet during a 10-d adjustment period. After the initial period, all dietary changes were made abruptly (i.e., overnight). This change was facilitated by the transfer of 6 to 8 L of rumen fluid from a cow that was consuming the LF diet to a cow that was starting a period on the LF diet. The LF diet was offered for the first time the night before the inoculation of rumen fluid to allow the lactic acid fermentation to begin before the addition of rumen fluid from an adapted cow. In addition, cows fed the LF diet were given fresh feed four times per day during the first 3 d to facilitate adaptation in the rumen. The strategy was successful, and cows fed the LF diets had minimal digestive disturbances.

Cows were milked at 0700 and 1900 h. Data on yield and composition of milk were collected during the last 4 d of each period. Milk samples were taken at each milking. One aliquot was stored at 4°C with a preservative (bronopol tablet; D&F Control System, Inc., San Ramon, CA) until analyzed for fat and protein content by infrared analysis (New York DHI, Ithaca, NY). An additional aliquot of milk was frozen daily and stored at –80°C until analyzed for fatty acids.

For fatty acid analysis, milk samples were pooled to form one composite sample per cow per period. Samples were extracted with a mixture of hexane and isopropanol (3:2, vol/vol), combined with methylacetate (1 ml/100 mg of lipid), and transesterified to fatty acid methyl esters in hexane using 1 *M* methanolic sodium methoxide freshly prepared from Fluka 71748 reagent (10). The turbid preparation was neutralized with oxalic acid and centrifuged; the residues of methanol were removed using $CaCl_2$. The analysis of methylesters was performed immediately on a gas chromatograph (Mega Series HRGC 5160; Carlo Erba Strumentazione, Milan, Italy) fitted with a flame ionization detector and equipped with an automatic injector (7673A; Hewlett Packard, Palo Alto, CA), split injection port (75 ml/min), and a 100-m fused silica capillary column (i.d., 0.25 mm) coated with 0.2 μm of CP-Sil 88 phase (Chrompack, Middelburg, The Netherlands). The injector and detector temperatures were 255°C, and the inlet pressure of H_2 carrier gas was 1.85 kg/cm^2. Each sample was run three times. First, the total fatty acid profile was determined using a temperature gradient program (70 to 240°C). Second, the oven was operated isothermally at 160°C to separate most of the *trans*-octadecenoic acids. Finally, the isothermal run was repeated by setting the oven temperature at 180°C to separate *trans*-13/14 (coelute as one peak) from the *cis*-9 peak. Complete separation of the *cis* and *trans* isomers

cannot be obtained through a single chromatographic run. However, the column and method used gave a very satisfactory separation of the major isomers of interest (Figure 1).

Fatty acid composition was expressed as a weight percentage of total fatty acids. Weight percentages of specific *trans*-octadecenoic acids were calculated as a proportion of total $C_{18:1}$ area (*trans*-4-$C_{18:1}$ through *trans*-16-$C_{18:1}$). The response correction factor for each fatty acid methyl ester, which was used to convert peak area percentage to weight percentage, was determined by analyzing butter oil of a known fatty acid composition (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium). Identification of the individual *trans* isomers was based on the *trans* isomer profile in the reference butter oil (CRM 164), published isomeric profiles (27, 49), and the use of *trans*-11 as a landmark isomer as suggested by Wolff and Bayard (49).

Blood samples (100 U of heparin/ml of blood) were obtained at hourly intervals for 12 h on the last day of each period. These samples were obtained via indwelling jugular catheters that had been inserted 24 h earlier. Plasma was harvested by centrifugation and stored at –20°C until analysis. Concentrations of glucose were determined by enzymatic colorimetric analysis using a commercial kit (Sigma Chemical Co., St. Louis, MO), and NEFA were determined by enzymatic colorimetric analysis (Wako Pure Chemical Industries, Osaka, Japan) as described by Sechen et al. (38). Insulin concentrations were determined by a double-antibody radioimmunoassay as previously described (26).

Rumen fluid samples (200 ml) were obtained by suction from six locations in the rumen liquid phase and composited nine times over a 12-h interval during the last day of each period. Rumen fluid was strained through four layers of cheesecloth, and pH was determined immediately. Rumen fluid samples were then centrifuged (20,000 × *g* at 4°C) for 20 min, and the supernatant was stored at –20°C until analysis. Volatile fatty acids were quantified by the method of Ehrlich et al. (13) using HPLC (Waters Chromatography Division, Millipore Corp., Milford, MA) with a Bio-Rad HPX-87H column (7.8 × 300 mm; Bio-Rad,

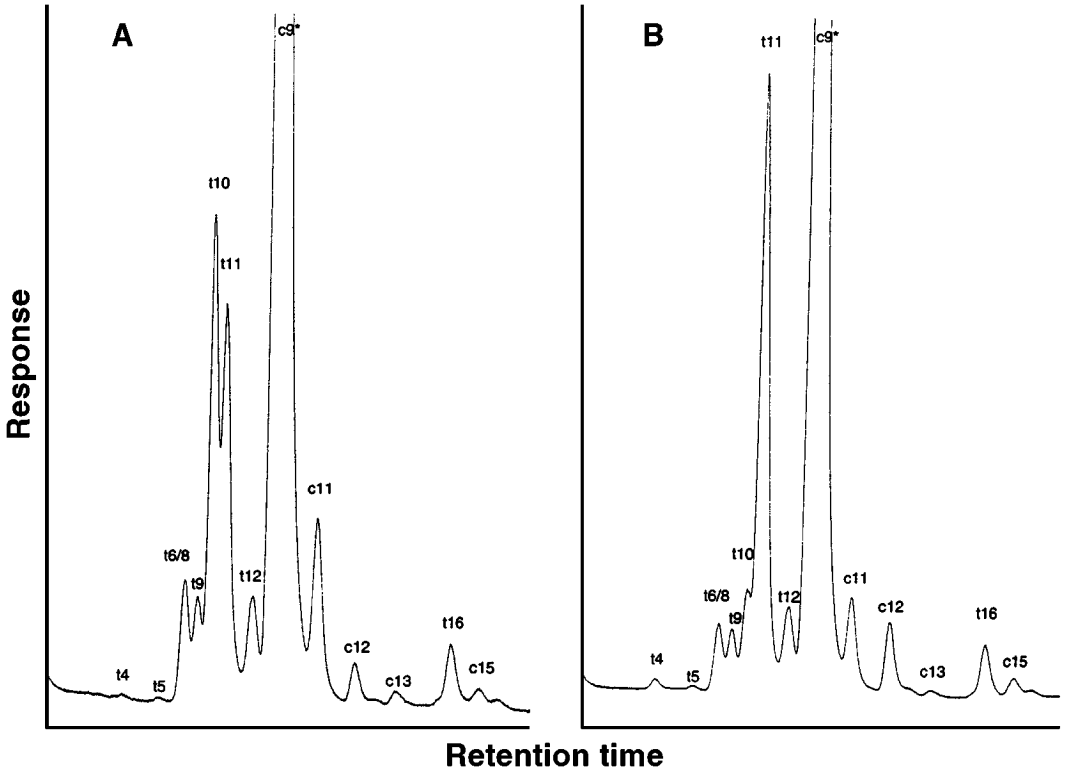

Figure 1. Chromatographs of milk fat (isothermal run at 160°C) that illustrate the separation of *cis* (c) and *trans* (t) isomers of octadecenoic acids. The y-axis represents arbitrary response units, and the x-axis represents the retention time from 40 to 52 min. Panel A represents milk fat from a cow fed the low fiber diet plus unsaturated fatty acids, and panel B represents milk fat from the same cow fed the high fiber diet plus unsaturated fatty acids. The asterisk for *cis*-9 highlights the coelution of *trans*-13/14 and *trans*-15 at this peak.

TABLE 1. Composition of total mixed diets.

Ingredient	Dietary composition[1] HF + SFA	HF + UFA	LF + SFA	LF + UFA
	(g/kg of DM)			
Chopped alfalfa hay	500	500	203	203
Degermed shelled corn	365	365	588	588
Soybean meal	120	120	177	177
Urea	5.0	5.0	10.0	10.0
Calcium phosphate	1.8	1.8	5.0	5.0
Limestone	2.8	2.8	10.4	10.4
Trace-mineralized salt and vitamins[2]	6.0	6.0	6.8	6.8
Fat addition	36	40	36	40

[1]High fiber (HF) diets had a forage to concentrate ratio of 50:50 and contained, on average, 88.8% DM, 20.0% CP, 19.2% ADF, 32.1% NDF, and 1.63 Mcal of NE_L/kg of DM. Low fiber (LF) diets had a forage to concentrate ratio of 20:80 and contained, on average, 87.8% DM, 21.0% CP, 9.1% ADF, 14.8% NDF, and 1.84 Mcal of NE_L/kg of DM. Fat additions were either corn oil as a source of unsaturated fatty acids (UFA) or a dry fat product (Energy Booster 100®; Milk Specialties Co., Dundee, IL) as a source of saturated fatty acids (SFA). Major fatty acids in the corn oil were 14% $C_{16:0}$, 2% $C_{18:0}$, 39% $C_{18:1}$, and 44% $C_{18:2}$. Major fatty acids in the dry fat product were 47% $C_{16:0}$, 36% $C_{18:0}$, 14% $C_{18:1}$, and 1% $C_{18:2}$.

[2]Contained 21 g/kg of NaCl and 3.4 g/kg of a trace mineral and vitamin mix. The trace mineral and vitamin mix contained (per kilogram of mix) 2.0 g of Zn, 0.97 g of Fe, 0.48 g of Cu, 0.044 g of I, 0.048 g of Co, 0.013 g of Se, 1,200,000 IU of vitamin A, 480,000 IU of vitamin D_3, and 4000 IU of vitamin E.

Richmond, CA) at 30°C, isocratic elution with 5 m*M* H_2SO_4, and UV detection at 210 nm. Quantification was on the basis of area, and a mixture of acetic, propionic, and butyric acids was included as a calibration standard at the beginning and end of each chromatographic run. Data within cow and within period were averaged for statistical analysis.

All cows completed the full Latin square. Data were analyzed by ANOVA, and treatment effects were tested using an *F* test. Single degree of freedom comparisons were made to evaluate the effects of fiber (LF vs. HF), the type of added fat (UFA vs. SFA), and the interaction of amount of fiber and type of fat.

RESULTS

The composition of the diets is presented in Table 1. To provide approximately equal amounts of fatty acids that differed in the degree of saturation, two treatments were used: corn oil was added at 4% of the diet (as fed), or a saturated fat product was added at 3.6% of the diet. Feed intakes are presented in Table 2 and were generally comparable across diets, which was consistent with the study design. However, intake of one cow was reduced by one-third during the 2nd wk each time the LF diet was fed, which affected the extent of rumen fermentation and MFD. Nevertheless, data from this cow are included, and the overall daily energy intakes for HF and LF diets averaged 38.1 and 36.2 Mcal of NE_L, respectively.

Effects of amount of fiber and type of fat added were significant for milk fat percentage and yield (Table 2). The addition of UFA to the LF diet resulted in the most pronounced depression of milk fat. In this case, milk fat percentage was 30% lower than that of cows fed the HF diet plus SFA. This

TABLE 2. Effect of amount of dietary fiber and type of added fat on performance.

Variable	Diet[1] HF + SFA	HF + UFA	LF + SFA	LF + UFA	SEM	Contrast Fiber	Fat	Interaction
						P		
Feed intake, kg/d	23.0	23.8	19.9	19.5	0.6	***	NS[2]	NS
Milk yield, kg/d	29.3	31.7	26.5	26.3	1.6	*	NS	NS
Milk fat								
%	3.58	3.36	3.33	2.49	0.16	**	*	†
kg/d	1.05	1.06	0.87	0.68	0.06	***	NS	NS
Milk protein								
%	3.01	3.07	3.10	3.24	0.12	NS	NS	NS
kg/d	0.87	0.97	0.82	0.85	0.03	*	NS	NS

[1]High fiber (HF) diets had a forage to concentrate ratio of 50:50, and low fiber (LF) diets had a forage to concentrate ratio of 20:80. Fat additions were either corn oil as a source of unsaturated fatty acids (UFA) or a dry fat product (Energy Booster 100®; Milk Specialties Co., Dundee, IL) as a source of saturated fatty acids (SFA).

[2]$P > 0.1$.

†$P < 0.1$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

result suggests a synergism of effects between the amount of fiber and the type of fat. Even with the variation in the response of cows to the LF diets, the interaction term approached significance for both milk fat percentage ($P = 0.09$) and milk fat yield ($P = 0.11$). Relative to the HF diet plus SFA, the addition of UFA to the HF diet or SFA to the LF diet resulted in a slightly lower (6 to 7%) milk fat percentage. Milk and milk protein yields decreased when the LF diets were fed, but yields were not affected by the type of added fat.

Rumen pH decreased significantly when LF diets were fed (Figure 2), and the pattern of VFA shifted to a higher proportion of propionate (Table 3). The type of added fat did not affect the pH, and the effects on the proportions of VFA were minimal. Circulating concentrations of insulin increased approximately twofold when the LF diets were fed, but concentrations were not affected by the type of fat in the diet. Plasma concentrations of glucose were highest, and those of NEFA were lowest, during the periods when cows were fed the LF diets (Table 3).

The addition of corn oil decreased the content of fatty acids in milk fat that are synthesized de novo (C_4 to C_{14}) as well as palmitic acid; octadecenoic acids, conjugated linoleic acid, and linoleic acid increased (Table 4). As for octadecenoic acid isomers, cows fed diets containing corn oil yielded milk fat with higher concentrations of total *cis* isomers ($P < 0.05$) and total *trans* isomers ($P < 0.001$). However, the concentrations of total *cis* and *trans* isomers of octadecenoic acid were not affected by dietary fiber ($P > 0.20$). The addition of corn oil also resulted in changes in the milk fat content of specific *cis* and *trans* isomers of octadecenoic acid (Table 4). Some of the most pronounced changes occurred in the *trans*-octadecenoic acids, and these isomers are profiled in Figure 3. The increase in *trans*-11-octadecenoic was particularly pronounced when corn oil was added to the diets. For the *trans* isomers, the most pronounced interaction between amount of fiber and type of fat occurred in the *trans*-10 content of milk fat ($P < 0.05$). Increase in the *trans*-10 isomer of cows fed the LF diets was marked only when UFA were added.

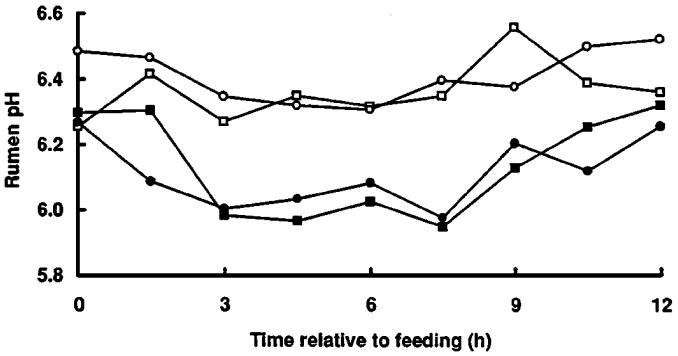

Figure 2. Effect of amount of dietary fiber and type of added dietary fat on rumen pH. Cows were fed immediately following sampling at time 0, and measurements were taken over a 12-h interval. Legend: high fiber diet (open symbols), low fiber diet (solid symbols), added saturated fatty acids (squares), and added unsaturated fatty acids (circles).

TABLE 3. Effect of amount of dietary fiber and type of added fat on rumen VFA and plasma concentrations of insulin and metabolites.

Variable	Diet[1] HP + SFA	HP + UFA	LF + SFA	LF + UFA	SEM	Contrast Fiber	Fat	Interaction
						P		
Rumen								
Total VFA, m*M*	97.1	90.9	98.8	93.4	4.9	NS[2]	NS	NS
Acetate, mmol/mol	672	665	581	564	17	***	*	NS
Propionate, mmol/mol	212	216	310	338	19	***	NS	NS
Butyrate, mmol/mol	101	99	104	88	10	NS	NS	NS
Plasma								
Insulin, ng/ml	1.4	1.5	3.0	2.7	0.4	**	NS	NS
Glucose, mg/dl	73.6	76.0	85.3	78.8	2.5	*	NS	NS
NEFA, μeq/L	119.7	137.6	103.5	101.9	6.8	**	NS	NS

[1]High fiber (HF) diets had a forage to concentrate ratio of 50:50, and low fiber (LF) diets had a forage to concentrate ratio of 20:80. Fat additions were either corn oil as a source of unsaturated fatty acids (UFA) or a dry fat product (Energy Booster 100®; Milk Specialties Co., Dundee, IL) as a source of saturated fatty acids (SFA).

[2]$P > 0.2$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

The yield of individual fatty acids in milk fat is presented in Table 5. Consistent with the difference in the composition of dietary fats, the addition of the dry fat product (SFA) increased the milk fat secretion of palmitic acid, and the addition of corn oil (UFA) increased the milk fat secretion of C_{18} fatty acids. Comparison with the diets supplemented with corn oil indicated that the MFD that occurred with the LF diet involved a reduction in the yield of both de novo synthesized fatty acids and those originating from the uptake of preformed fatty acids. The yield of de novo fatty acids was reduced by 32% (283 g/d for cows fed the HF diet plus UFA vs. 192 g/d for cows fed the LF diet plus UFA; C_4 to C_{14} plus one-half $C_{16:0}$), and the yield of preformed fatty acids was reduced by 42% (592 g/d for cows fed the HF diet plus UFA vs. 343 g/d for cows fed the LF diet plus UFA; one-half $C_{16:0}$ plus C_{18} fatty acids). These values are similar to the overall reduction of 36% in milk fat yield observed for cows fed the LF diet plus UFA compared with that of cows fed the HF diet plus UFA (Table 2).

DISCUSSION

Theories involving MFD in lactating ruminants can be broadly summarized into two categories: those that attribute MFD to a direct inhibition of mammary gland synthesis of milk fat (12, 14) and those that consider MFD to be a consequence of a shortage in the supply of lipid precursors to the mammary gland (1, 43, 47). A number of compounds that can be derived from the diet or produced by rumen fermentation or animal metabolism have been suggested as possible effectors that could inhibit milk fat synthesis in the mammary gland. These compounds include partially hydrogenated fatty acids or, more specifically, *trans*-octadecenoic acids, methylmalonic acid, and cyclopropene fatty acids (i.e., sterculic acid) (2, 7, 14, 31). Direct inhibition of milk fat synthesis by *trans*-octadecenoic acids was first proposed more than two decades ago (12). Pennington and Davis (31) further speculated that *trans*-octadecenoic acids, resulting from the partial hydrogenation of UFA in the rumen,

TABLE 4. Effect of amount of dietary fiber and type of added fat on fatty acid composition of milk.

	Diet[1]					Contrast		
Profile	HF + SFA	HF + UFA	LF + SFA	LF + UFA	SEM	Fiber	Fat	Interaction
	(g/100 g of fatty acids)					*P*		
C_4 to C_{14}	22.0	19.9	23.3	20.9	0.66	NS[2]	*	NS
$C_{16:0}$	32.3	19.7	34.0	22.6	1.17	NS	***	NS
$C_{18:0}$	10.0	14.5	8.5	10.1	1.44	†	†	NS
$C_{18:1}$	23.1	33.9	21.7	31.4	1.53	NS	***	NS
$C_{18:1}$ Isomers								
trans-4	0.01	0.05	0.01	0.01	0.01	NS	NS	NS
trans-5	<0.01	0.02	<0.01	0.01	0.01	NS	†	NS
trans-6/8	0.17	0.49	0.16	0.66	0.04	NS	***	†
trans-9	0.13	0.43	0.13	0.55	0.07	NS	**	NS
trans-10	0.33	0.70	0.42	2.90	0.43	*	*	*
trans-11	0.63	4.53	0.57	2.52	0.48	†	***	†
trans-12	0.27	0.81	0.24	0.57	0.06	†	***	NS
trans-13/14 + *cis*-6	0.29	0.86	0.28	0.70	0.08	NS	***	NS
cis-9 + *trans*-15	19.98	23.57	18.68	21.28	1.51	NS	†	NS
cis-11	0.77	1.03	0.80	1.24	0.06	NS	***	NS
cis-12	0.11	0.62	0.09	0.30	0.04	**	***	*
cis-13	0.07	0.10	0.05	0.12	0.01	NS	*	NS
cis-14 + *trans*-16	0.13	0.55	0.12	0.31	0.06	†	*	†
CLA[3]	0.35	1.98	0.33	1.10	0.21	†	***	†
$C_{18:2}$	1.65	2.13	1.78	4.03	0.46	†	*	†
$C_{18:3}$	0.30	0.23	0.18	0.20	0.03	*	NS	†

[1]High fiber (HF) diets had a forage to concentrate ratio of 50:50, and low fiber (LF) diets had a forage to concentrate ratio of 20:80. Fat additions were either corn oil as a source of unsaturated fatty acids (UFA) or a dry fat product (Energy Booster 100®; Milk Specialties Co., Dundee, IL) as a source of saturated fatty acids (SFA).

[2]$P > 0.2$.

[3]Conjugated linoleic acid (*cis*-9, *trans*-11 isomer).

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

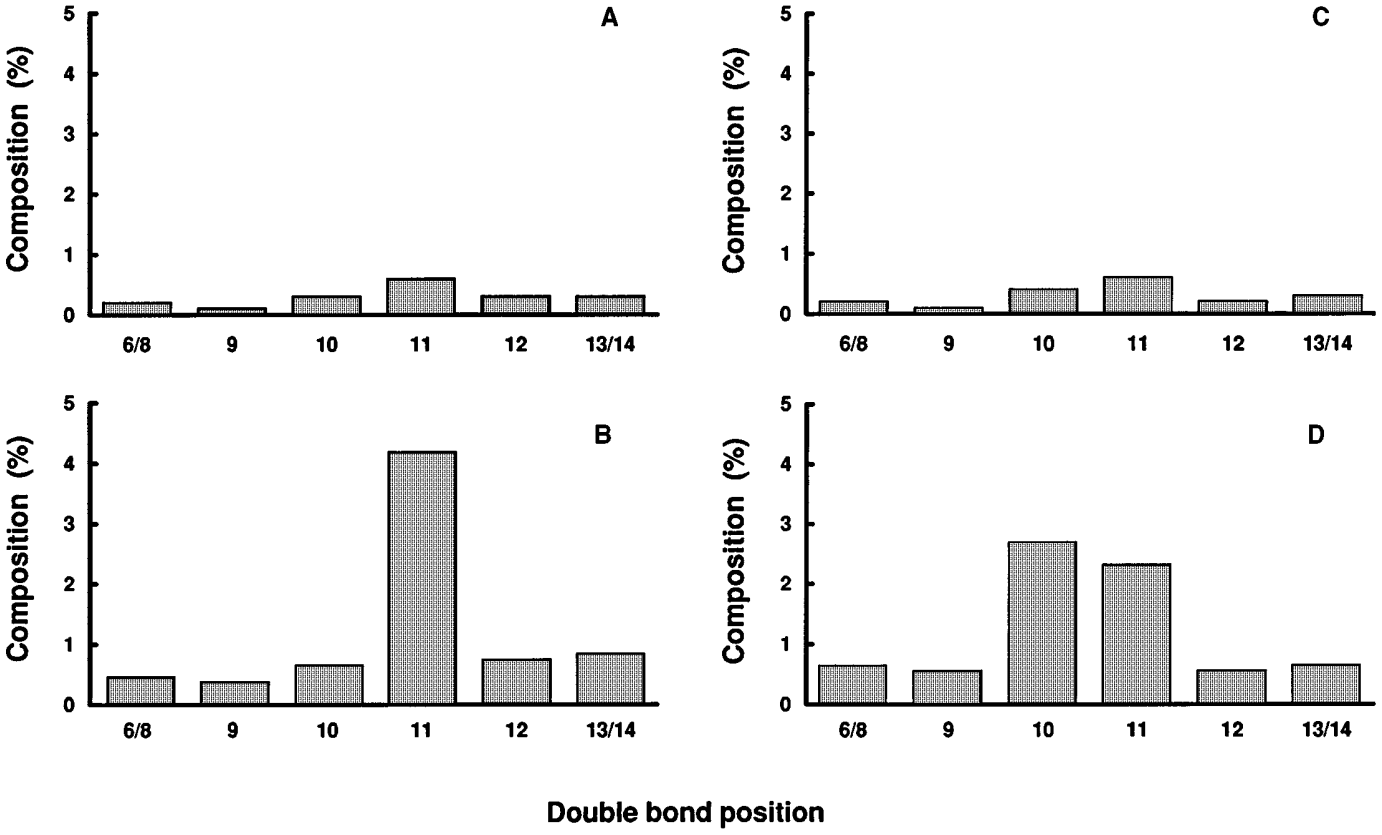

Figure 3. Effect of amount of dietary fiber and type of added fat on the profile of *trans*-octadecenoic acid isomers in milk fat. Unsaturated fatty acids (UFA) or saturated fatty acids (SFA) were added to the high fiber (HF) and low fiber (LF) diets. Panel A = HF diet plus SFA, panel B = HF diet plus UFA, panel C = LF diet plus SFA, and panel D = LF diet plus UFA. Statistical analysis is shown in Table 4.

TABLE 5. Effect of amount of dietary fiber and type of added fat on fatty acid yield of milk.

	Diet[1]					Contrast		
Profile	HF + SFA	HF + UFA	LF + SFA	LF + UFA	SEM	Fiber	Fat	Interaction
	(g/d of yield)					*P*		
C_4 to C_{14}	206	189	188	125	13	*	*	NS[2]
$C_{16:0}$	301	187	269	133	14	*	***	NS
$C_{18:0}$	93	136	65	63	13	**	NS	NS
$C_{18:1}$	201	255	153	144	17	**	NS	NS
trans-$C_{18:1}$ Isomers	14	66	12	40	4	*	***	*
CLA[3]	3	19	2	7	2	**	***	*
$C_{18:2}$	15	20	14	21	1	NS	***	NS
$C_{18:3}$	3	2	1	1	1	***	†	NS

[1]High fiber (HF) diets had a forage to concentrate ratio of 50:50, and low fiber (LF) diets had a forage to concentrate ratio of 20:80. Fat additions were either corn oil as a source of unsaturated fatty acids (UFA) or a dry fat product (Energy Booster 100®; Milk Specialties, Co., Dundee, IL) as a source of saturated fatty acids (SFA).

[2]$P > 0.2$.

[3]Conjugated linoleic acid (*cis*-9, *trans*-11 isomer).

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

were involved in MFD when high concentrate diets or polyunsaturated oils were fed. Recently, two elegant studies (16, 50) have demonstrated that *trans*-octadecenoic acids produced in the rumen or infused postruminally were associated with depressed milk fat synthesis.

The hypothesis for the current study was that two conditions are necessary for MFD: the presence of dietary UFA and an altered rumen environment that leads to an incomplete hydrogenation and production of *trans*-octadecenoic acids. Cows were fed a total mixed diet at two amounts of fiber to alter the rumen environment (forage to concentrate ratios of 50:50 or 20:80). The basal diet was formulated to be low in fat, and the added fat was either corn oil as a source of UFA or a dry fat product, which was mostly SFA. The LF diet contained sufficient amounts of readily fermentable carbohydrates relative to fiber to result in marked changes in rumen fermentation. Total VFA concentrations were not altered when the LF diets were fed, but the molar percentage of propionate increased to well above 25% (Table 3), a level that has been considered to be associated with a decrease in milk fat content (51). Although propionate production was not measured in the present study, rumen concentration was increased by over 50% when cows were fed the LF diets (Table 3). Earlier studies (5, 24) have shown that the rumen concentration of propionate is highly correlated with the rate of production. Rumen pH was lower when cows were fed the LF diets (Figure 2), which is consistent with the high dietary starch and low dietary NDF content (34, 36).

Amount of fiber in the diet and type of fat had significant effects on milk fat percentage and yield. Effects were most pronounced when corn oil was added to the LF diet. In this case, milk fat percentage and yield were 30 and 35% lower, respectively, than those of cows fed the HF diet plus SFA (Table 2). Compared with the other diets, the effects of type of fat and the ratio of forage to concentrate appeared to be more than additive, and, despite variation in the intake responses of the cows, the interaction approached significance for both fat percentage (P = 0.09) and fat yield (P = 0.11) (Table 2). Thus, results support the hypothesis that both an altered rumen environment and the presence of UFA in the diet are necessary conditions for a substantial decrease in the percentage and yield of milk fat.

To examine the specific role of *trans*-octadecenoic acids in MFD, we summarized the available data related to milk fat content of *trans*-octadecenoic acids and MFD (Figure 4). The relationship between the change in *trans*-octadecenoic content in milk fat and the change in milk fat percentage remained consistent across dietary comparisons involving 17 studies (3, 4, 9, 16, 21, 22, 23, 30, 31, 33, 35, 39, 40, 41, 42, 44, 50) and comparisons from 27 individual treatments (Figure 4A). The relationship is impressive because it exists over substantial changes in *trans*-octadecenoic acid percentages and across a wide range of feeding situations. Diets used in those studies, all of which observed a reduction in milk fat percentage, included LF diets and diets supplemented with partially hydrogenated oils, plant oils, fish oils, or full fat oilseeds. The exclusion of diets with added free oils (Figure 4B) improved the R^2 (0.82 vs. 0.54). In the present study, the sum of *trans*-6- through *trans*-12-octadecenoic acids was 1.5, 7.0, 1.5, and 7.2% of total fatty acids for cows fed the HF diet plus SFA, the HF diet plus UFA, the LF diet plus SFA, and the LF diet plus UFA, respectively (SEM = 0.44). A significant increase occurred in the milk fat content of

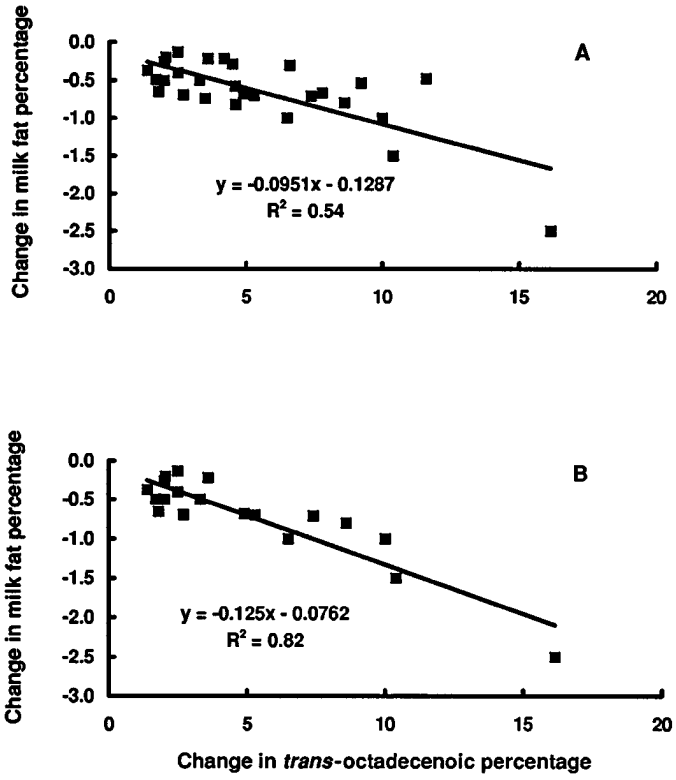

Figure 4. Relationship between the change in *trans*-octadecenoic acid content of milk fat and the change in milk fat percentage. Panel A includes dietary comparisons involving 17 studies (3, 4, 9, 16, 21, 22, 23, 30, 31, 33, 35, 39, 40, 41, 42, 44, and 50) and 27 individual treatments. Panel B excludes dietary treatments that involved the addition of unsaturated oils; the remaining comparisons represented 13 studies and 19 individual treatments.

trans-octadecenoic acid when UFA were added to the diet ($P < 0.001$). However, alterations in rumen fermentation caused by the dietary differences in the amount of fiber had no effect on the amount of *trans*-octadecenoic acids in milk fat, and there was no interaction of type of fat and amount of dietary fiber ($P > 0.2$). Thus, the total amount of *trans*-octadecenoic acids did not correspond to changes in milk fat percentage that we expected.

Based on these results and those in Figure 4, we postulated that the degree of MFD might vary in relation to proportions of different *trans* isomers of octadecenoic acid. When we expanded the analysis to examine the isomeric profile of the *trans*-octadecenoic acid, substantial differences were observed among the dietary treatments (Figure 3). Although the addition of UFA resulted in marked increases in the content of *trans*-11-octadecenoic acid in milk fat for cows fed both LF and HF diets, only the LF diet plus UFA resulted in an increased content of *trans*-10-octadecenoic acid in milk fat and a significant decrease in milk fat percentage. We observed the same close relationship between MFD and an increase in the content of *trans*-10-octadecenoic acid in milk fat in a study that examined changes in the profile of *trans*-octadecenoic acids associated with MFD that was induced by a high concentrate, LF diet (17). Thus, there may be specific *trans* isomers of octadecenoic acids or related metabolites [e.g., isomers of conjugated linoleic acid (17)] that are associated more closely with the changes in milk fat synthesis than were observed for the total amount of *trans*-octadecenoic acid or the amount of the *trans*-11 isomer, the major isomer produced in the rumen. In particular, the increase in the *trans*-10 isomer of octadecenoic acid appeared to be a consistent marker of MFD induced by the LF diet, and it will be interesting to extend these measurements to other dietary situations that cause MFD. The milk fat content and yield of conjugated linoleic acid (*cis*-9, *trans*-11 isomer) were significantly increased when corn oil was added to the diet (Tables 4 and 5). However, the increase was less pronounced for cows fed the LF diet (significant interaction). The difference in response can be partly explained by the formation of other conjugated linoleic acid isomers in the rumen and their appearance in milk fat when LF diets were fed (data not presented).

Davis and Brown (12) speculated that the physical or chemical nature of the *trans*-octadecenoic acids in triacylglycerols might affect their uptake or use in milk fat synthesis. However, Bickerstaffe et al. (8) demonstrated that rates of absorption from the small intestine, transfer into lymph, uptake by the mammary gland, and appearance in milk fat were similar for *cis* and *trans* isomers of octadecenoic acid. Mammary extraction of *trans*-octadecenoic acids from triacylglycerols was found to be similar to the extraction of stearic acid ($C_{18:0}$) but higher than the extraction of the respective *cis*-monoenoic acid (46). The physical and chemical characteristics of *trans*-octadecenoic acids, which are monounsaturated but with a melting point similar to that of saturated stearic acid, may be important in the mechanism of milk fat inhibition (19). Our data raise the possibility that the position of the double bond may be an important determinant of the inhibitory effect. A likely site for the inhibitory effect on milk fat synthesis is diacylglycerol acyltransferase, the putative rate limiting enzyme in triacylglycerol synthesis (25). However, other important steps, such as de novo fatty acid synthesis, Δ-9-desaturase activity, and the translocation and secretion of the milk fat, are also potential sites for the inhibition of milk fat synthesis.

Altered rumen fermentation with a decreased ratio of acetate to propionate is characteristic in many feeding situations that result in depressed milk fat (12, 43, 47). Based on these changes it was initially thought that MFD might be related to a shortage of acetate or βHBA. However, studies have shown that the production of these VFA in the rumen was unaltered (5, 11, 29) and that endogenous acetate production was relatively constant under a variety of circumstances (32). A shortage of lipogenic precursors for mammary synthesis of milk fat was also the basis of the glucogenic insulin theory of MFD. According to that theory, the circulating insulin that is increased when high concentrate diets are fed was postulated to cause the preferential channeling of lipid precursors to adipose tissue, away from the mammary gland, thereby reducing milk fat synthesis (1, 12, 43, 47). In the present study, the circulating concentrations of insulin, glucose, and NEFA differed according to the ratios of forage to concentrate in the diet (Table 3), and the effects were consistent with general observations relative to LF diets, which show a tendency toward MFD in lactating dairy cows (6, 12, 43). However, these differences were not parallel to changes in milk fat percentage. In particular, we observed elevated insulin concentrations when the LF diet was fed, regardless of the type of fat supplement, but a marked MFD only occurred when the LF diet plus UFA was fed (Table 2). Other studies have also resulted in no MFD when circulating insulin concentrations were chronically elevated by insulin injection (37, 45) or by hyperinsulinemic-euglycemic clamp

(18, 26). Together, these data suggest that the enhanced uptake of lipid precursors by adipose tissue during MFD may be a consequence rather than a cause of reduced mammary gland use of lipogenic precursors.

CONCLUSIONS

The overall results from the present study demonstrate that two conditions are necessary for MFD: the presence of rumen substrate in the form of dietary UFA and an altered rumen environment that leads to incomplete biohydrogenation. Our results also support the hypothesis of Davis et al. (12, 31) that *trans*-octadecenoic acids, produced by incomplete biohydrogenation in the rumen, are involved in MFD, which is induced by LF diets. However, rather than being associated with total *trans*-octadecenoic acids, we showed that MFD might vary according to the proportions of specific *trans*-octadecenoic isomers or related metabolites. Marked MFD was most clearly associated with an increase in the milk fat content of the *trans*-10 isomer.

ACKNOWLEDGMENTS

The skilled assistance of Bill English, Dan Smith, Christina Winter, and Dottie Ceurter is appreciated. The authors also acknowledge Milk Specialties Inc. for the donation of the dry fat product used in the study.

REFERENCES

1 Annison, E. F. 1976. Energy utilisation in the body. Pages 169–199 *in* Principles of Cattle Production. H. Swan and W. H. Broster, ed. Butterworths, London, England.
2 Astrup, H. N., L. Vik-Mo, A. Ekern, and F. Bakke. 1975. Feeding protected and unprotected oils to dairy cows. J. Dairy Sci. 59:426–430.
3 Banks, W., J. L. Clapperton, A. K. Girdler, and W. Steele. 1984. Effect of inclusion of different forms of dietary fatty acid on the yield and composition of cow's milk. J. Dairy Res. 51:387–395.
4 Banks, W., J. L. Clapperton, M. E. Kelly, A. G. Wilson, and J. M. Crawford. 1980. The yield, fatty acid composition and physical properties of milk fat obtained by feeding soya oil to dairy cows. J. Sci. Food Agric. 31:368–374.
5 Bauman, D. E., C. L. Davis, and H. F. Bucholtz. 1971. Propionate production in the rumen of cows fed either a control or high grain, low fiber diet. J. Dairy Sci. 54:1282–1287.
6 Bell, A. W. 1980. Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. Prog. Lipid Res. 18: 117–164.
7 Bickerstaffe, R., and A. R. Johnson. 1972. The effect of intravenous infusions of sterculic acid on milk fat synthesis. Br. J. Nutr. 27:561–570.
8 Bickerstaffe, R., D. E. Noakes, and E. F. Annison. 1972. Quantitative aspects of fatty acid biohydrogenation, absorption and transfer into milk fat in the lactating goat, with special reference to the *cis*- and *trans*-isomers of octadecanoate and linoleate. Biochem. J. 130:607–617.
9 Casper, D. F., D. J. Schingoethe, R. P. Middaugh, and R. J. Baer. 1988. Lactational responses of dairy cows to diets containing regular and high oleic acid sunflower seeds. J. Dairy Sci. 71: 1267–1274.
10 Christie, W. W. 1982. A simple procedure of rapid transmethylation of glycerolipids and cholesteryl esters. J. Lipid Res. 23: 1072–1075.
11 Davis, C. L. 1967. Acetate production in the rumen of cows fed either control or low fiber, high grain diets. J. Dairy Sci. 50: 1621–1625.
12 Davis, C. L., and R. E. Brown. 1970. Low-fat milk syndrome. Pages 545–565 *in* Digestion and Metabolism in the Ruminant. A. T. Phillipson, ed. Oriel Press, Newcastle upon Tyne, England.
13 Ehrlich, G. G., D. F. Goerlitz, J. H. Bourel, G. V. Eisen, and E. M. Godsy. 1981. Liquid chromatographic procedure for fermentation product analysis in the identification of anaerobic bacteria. Appl. Environ. Microbiol. 42:878–886.
14 Frobish, R. A., and C. L. Davis. 1977. Theory involving proportionate and vitamin B_{12} in the low milk fat syndrome. J. Dairy Sci. 60:268–273.
15 Funk, D. A. 1993. Optimal genetic improvement for the high producing herd. J. Dairy Sci. 76:3278–3286.
16 Gaynor, P. J., R. A. Erdman, B. B. Teter, J. Sampugna, A. V. Capuco, D. R. Waldo, and M. Hamosh. 1994. Milk fat yield and composition during abomasal infusion of *cis* or *trans* octadecenoates in Holstein Cows. J. Dairy Sci. 77:157–165.
17 Griinari, J. M., P. Y. Chouinard, and D. E. Bauman. 1997. *Trans* fatty acid hypothesis of milk fat depression revised. Pages 208–216 *in* Cornell Nutr. Conf. Feed Manuf., Rochester, NY. Cornell Univ., Ithaca, NY.
18 Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, and D. L. Palmquist. 1997. Role of insulin in the regulation of milk fat synthesis in dairy cows. J. Dairy Sci. 80:1076–1084.
19 Hagemeister, H. 1990. Fetteinsatz in der Milchviehfutterung unter besonderer Berucksichtigung der im Pansen entstehenden *trans*-Fettsauren. Kieler Milch. Forsch. 42:271–280.
20 Harfoot, C. G., and G. P. Hazelwood. 1988. Lipid metabolism in the rumen. Pages 285–322 *in* The Rumen Microbial Ecosystem. P. N. Hobson, ed. Elsevier Sci. Publ. Ltd., London, England.
21 Jenkins, T. C., H. G. Bateman, and S. M. Block. 1996. Butylsoyamide increases unsaturation of fatty acids in plasma and milk of lactating dairy cows. J. Dairy Sci. 79:585–590.
22 Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of dietary forage concentration and buffer addition on duodenal flow of *trans*-$C_{18:1}$ fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2104–2114.
23 Kennelly, J. J. 1987. Full-fat canola seed for lactating dairy cows. Pages 257–262 *in* 8th Prog. Rep. Res. on Canola Seed, Oil, Meal and Meal Fractions. Canola Counc. Canada, Ottawa, ON, Canada.
24 Leng, R. A. 1970. Formation and production of volatile fatty acids in the rumen. Pages 406–421 *in* Digestion and Metabolism in the Ruminant. A. T. Phillipson, ed. Oriel Press, Newcastle upon Tyne, England.
25 Mayorek, N., I. Grinstein, and J. Bar-Tana. 1989. Triacylglycerol synthesis in cultured rat hepatocytes. The rate limiting role of diacylglycerol acyltransferase. Eur. J. Biochem. 182: 395–400.
26 McGuire, M. A., J. M. Griinari, D. A. Dwyer, and D. E. Bauman. 1995. Role of insulin in the regulation of mammary synthesis of fat and protein. J. Dairy Sci. 78:816–824.
27 Molkentin, J., and D. Precht. 1995. Optimized analysis of *trans*-octadecenoic acids in edible fats. Chromatographia 41:267–272.
28 National Research Council. 1988. Designing Foods. Natl. Acad. Sci., Washington, DC.
29 Palmquist, D. L., C. L. Davis, R. E. Brown, and D. S. Sachan. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into mil fat of D(-)betahydroxybutyrate. J. Dairy Sci. 52:633–638.
30 Palmquist, D. L., and F. L. Schanbacher. 1991. Dietary fat

composition influences fatty acid composition of milk fat globule membrane in lactating cows. Lipids 26:718–722.
31 Pennington, J. A., and C. L. Davis. 1975. Effects of intraruminal and intraabomasal additions of cod liver oil on milk fat production in the cow. J. Dairy Sci. 58:49–55.
32 Pethick, D. W., D. B. Lindsay, P. J. Barker, and A. J. Northrop. 1981. Acetate supply and utilization by the tissues of sheep in vivo. Br. J. Nutr. 46:97–110.
33 Piperova, L. S., B. B. Teter, and R. A. Erdman. 1995. Time course of dietary milk fat depression in lactating dairy cows. J. Dairy Sci. 78(Suppl. 1):218.(Abstr.)
34 Pitt, R. E., J. S. Van Kessel, D. G. Fox, A. N. Pell, M. C. Barry, and P. J. Van Soest. 1996. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. J. Anim. Sci. 74:226–244.
35 Romo, G., D. Casper, R. Erdman, and B. B. Teter. 1996. Abomasal infusion of *cis* and *trans* fatty acid isomers and energy metabolism of lactating dairy cows. J. Dairy Sci. 79: 2005–2015.
36 Russell, J. B., and J. M. Chow. 1993. Another theory for the action of ruminal buffer salts: decreased starch fermentation and propionate production. J. Dairy Sci. 76:826–830.
37 Schmidt, G. H. 1966. Effect of insulin on yield and composition of milk of dairy cows. J. Dairy Sci. 49:381–385.
38 Sechen, S. J., F. R. Dunshea, and D. E. Bauman. 1990. Somatotropin in lactating cows: effect on response to epinephrine and insulin. Am. J. Physiol. 258:E582–E588.
39 Selner, D. R., and L. H. Schultz. 1980. Effects of feeding oleic acid or hydrogenated vegetable oils to lactating cows. J. Dairy Sci. 63:1235–1241.
40 Stegeman, G. A., D. P. Casper, D. J. Schingoethe, and R. J. Baer. 1992. Lactational responses of dairy cows fed unsaturated dietary fat and receiving bovine somatotropin. J. Dairy Sci. 75: 1936–1945.
41 Storry, J. E., and J.A.F. Rook. 1965. The effects of a diet low in hay and high in flaked maize on milk fat secretion and on the concentration of certain constituents in the blood plasma. Br. J. Nutr. 19:101–109.
42 Sundstol, F. 1974. Hydrogenated marine fat as feed supplement. Pages 1–50 *in* Sci. Rep. Agric. Univ. Norway, Dep. Anim. Nutr. Vol. 53, No. 25. Agric. Univ. Norway, Ås, Norway.
43 Sutton, J. D. 1989. Altering milk composition by feeding. J. Dairy Sci. 72:2801–2814.
44 Teter, B. B., J. Sampugna, M. Keeney, and J. Vandersall. 1989. Effects of fish meal feeding on milk and rumen fatty acid composition. J. Dairy Sci. 67(Suppl. 1):549.(Abstr.)
45 Thomas, P. C., D. G. Chamberlain, P. A. Martin, and S. Robertson. 1987. Dietary energy intake and milk yield and composition in dairy cows. Pages 188–191 *in* Energy Metabolism of Farm Animals. P. W. Moe, H. F. Tyrrell, and P. J. Reynolds, ed. Rowman and Littlefield, Totowa, NJ.
46 Thompson, G. E., and W. W. Christie. 1991. Extraction of plasma triacylglycerols by the mammary gland of the lactating cow. J. Dairy Res. 58:251–255.
47 Van Soest, P. J. 1963. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency. A review. J. Dairy Sci. 46:204–216.
48 Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell Univ. Press, Ithaca, NY.
49 Wolff, R. L., and C. C. Bayard. 1995. Improvement in the resolution of individual *trans*-18:1 isomers by capillary gas liquid chromatography: use of a 100 m CP-Sil 88 column. J. AOCS 72:1197–1201.
50 Wonsil, B. J., J. H. Herbein, and B. A. Watkins. 1994. Dietary and ruminally derived *trans*-18:1 fatty acids alter bovine milk lipids. J. Nutr. 124:556–565.
51 Woodford, J. A., N. A. Jorgensen, and G. P. Barrington. 1986. Impact of dietary fiber and physical form on performance of lactating dairy cows. J. Dairy Sci. 60:1035–1047.