Milk Fat Yield and Composition During Abomasal Infusion of *Cis* or *Trans* Octadecenoates in Holstein Cows[1]

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ABSTRACT

The role of *trans*-$C_{18:1}$ fatty acids in milk fat depression was examined. Six rumen-cannulated Holstein cows were assigned to two Latin squares with 21-d periods. The common basal diet contained 40% forage and 60% concentrate. Treatments were the uninfused control, 750 g/d of a mixture of *cis* fat (65% high oleic sunflower oil and 35% cocoa butter), and 750 g/d of a mixture of *trans* fat (93% shortening and 7% corn oil) infused into the abomasum via a tube that passed through the rumen cannula. Milk yield was similar among treatments. Milk fat percentage and yield were lower, and milk citrate concentration was higher, for the *trans* than the *cis* treatment. Changes in the fatty acid composition of milk were similar for the *cis* and *trans* treatments compared with the control except for *trans*-$C_{18:1}$. The concentration of *trans*-$C_{18:1}$ was greater for the *cis* and *trans* treatments than for the control and was greater for the *trans* than for the *cis* treatment. These data clearly demonstrated that infusion of *trans*-$C_{18:1}$ fatty acids into the abomasum depressed milk fat percentage and yield. We speculate that reduced synthesis of fatty acids and reduced activity of acyl transferase in mammary tissue contributed to depressed milk fat percentage for the *trans* treatment.

(**Key words**: *trans*-$C_{18:1}$ fatty acids, fat depression, lipoprotein lipase, milk citrate)

Abbreviation key: **FA** = fatty acid, **LPL** = lipoprotein lipase, **TG** = triacylglyceride.

INTRODUCTION

Dietary fat changes the fatty acid (**FA**) composition of milk and, in some cases, may depress milk fat yield (7, 14, 32). Various hypotheses relate structural and configurational differences of dietary FA to changes in milk FA composition (7, 14, 32). This study pursued the hypothesis that *trans*-$C_{18:1}$ FA contribute to milk fat depression.

This hypothesis is supported by the observation that the concentration of *trans*-$C_{18:1}$ FA in milk increases when milk fat percentage decreases (11, 29, 33). Because bacteria in the rumen biohydrogenate dietary long-chain polyunsaturated FA to produce *trans*-$C_{18:1}$ FA (18), diets containing a high percentage of concentrate or unsaturated fats would likely increase the production of *trans*-$C_{18:1}$ FA in the rumen.

The arrangement of carbon and hydrogen atoms around the double bond in *trans*-$C_{18:1}$

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FA results in a nearly straight-chain configuration, closely resembling a saturated FA. The enzyme stearoyl-coenzyme A desaturase catalyzes the conversion of $C_{18:0}$ to *cis*-$C_{18:1}$ FA in mammary tissue. Substitution of *trans*-$C_{18:1}$ for $C_{18:0}$ FA may depress the activity of this enzyme, thereby altering the FA composition and physical properties of triacylglyceride (TG) in milk (19, 35).

In addition, hepatic and mammary tissues utilize geometric isomers of long-chain FA at different rates. For example, Okuyama et al. (24) demonstrated that hepatic microsomes esterified *trans*-$C_{18:1}$ FA at twice the rate of *cis*-$C_{18:1}$ FA to the *sn*-1 position of *sn*-glyceryl-3-phosphorylcholine. In contrast, Askew et al. (2) demonstrated that mammary acyl transferases esterified *cis*-$C_{18:1}$ FA at a greater rate than *trans*-$C_{18:1}$ FA in diacylglyceride and TG synthesis. Thompson and Christie (34) reported mammary extraction ratios of 43% for *cis*-$C_{18:1}$ FA compared with 74% for *trans*-$C_{18:1}$ FA.

The relationship between *trans*-$C_{18:1}$ FA and milk fat has not been clearly defined.Therefore, the objective of this study was to compare the effects of infusion of *cis*-$C_{18:1}$ and *trans*-$C_{18:1}$ FA into the abomasum on milk fat synthesis and composition in high producing cows.

TABLE 1. Ingredient and chemical composition of the basal diet.

	Dry basis	
	(%)	
Ingredients	$\bar{X}$	SE
Corn silage	22.9	
Alfalfa haylage	17.5	
Soybean meal	23.0	
Ground corn	30.3	
Meat and bone meal	3.3	
Sodium bicarbonate	1.2	
Limestone	.6	
Trace-mineralized salt	.5	
Dynamate®[1]	.6	
Vitamin A, D, E premix[2]	.1	
Chemical analysis		
Samples analyzed, no.	6	
CP, %	23.4	.3
NDF, %	25.4	.7
ADF, %	15.6	.6
Ca, %	.88	.03
P, %	.46	.01
Mg, %	.25	.01
K, %	1.26	.03
DM, %	50.6	.8
NE_L, Mcal/kg DM	1.67	
Estimated RUP,[3] % of DM	7.6	

[1]Pitman-Moore, Inc., Chicago, IL; guaranteed analysis: 22% S, 18% K, 11% Mg.

[2]Vitamins A, D, and E present at 2,059,310, 453,592, and 227 IU/kg of premix, respectively.

[3]Rumen-undegradable protein.

MATERIALS AND METHODS

Cows, Diet, and Treatments

Six multiparous rumen-cannulated Holstein cows averaging 55 DIM were assigned randomly to treatment sequences balanced for carry-over effects in two 3 × 3 Latin squares. Cows were housed in a tie-stall barn and milked at 0630 and 1930 h daily.

A single diet (40% forage, 60% concentrate, DM basis) was offered for ad libitum intake throughout the 9-wk study (Table 1). Orts were removed at 0500 h, and cows were fed at 0730 h.

One fat mixture contained 65% high oleic sunflower oil (SVO Enterprises, East Lake, OH) and 35% cocoa butter (Wilbur Chocolate Co., Lititz, PA) and is referred to as the *cis* treatment. The other fat mixture contained 93% shortening (Auth Brothers, Washington, DC) and 7% corn oil (Continental Smelkinson, Jessup, MD) and is referred to as the *trans* treatment. Proportions of fat from each source were chosen to equalize the quantities of as many FA as possible except for *cis*- and *trans*-$C_{18:1}$ (Table 2). Fats were mixed and preweighed (755 g per container) every 2 wk and stored at 10 to 15°C. Containers were placed in an incubator at 37°C to liquefy fat within 24 h of abomasal infusion. Abrupt changes in the treatment that was administered to each cow occurred on d 1 of each 21-d experimental period.

Experimental Procedure

Fat was infused via Tygon® tubing (.48 cm i.d., .64 cm o.d.; VWR Scientific, Bridgeport, NJ) that passed through a small opening in the rumen cannula plug through the omasum into the abomasum. The tubing was maintained in

TABLE 2. Fatty acid composition of fat mixtures infused into the abomasum.

	cis[1]		*trans*[2]	
	$\bar{X}$	SE	$\bar{X}$	SE
Samples analyzed, no.	21		24	
Fatty acids, g/100 g of fat				
$C_{16:0}$	12.3	.2	13.6	.2
$C_{18:0}$	15.2	.3	15.8	.3
cis-$C_{18:1}$	64.7	.2	21.1	1.1
trans-$C_{18:1}$[3]	ND[4]		43.2	.5
$C_{18:2}$	7.2	.2	5.5	.2
Others	.6	.2	.8	.6

[1]*cis* = Mixture of 65% high oleic sunflower oil and 35% cocoa butter.

[2]*trans* = Mixture of 93% vegetable shortening and 7% corn oil.

[3]Includes all *trans*-$C_{18:1}$ fatty acid isomers.

[4]Not detected.

the abomasum with a plastisol flange cemented 2 cm from the distal end (8 cm × .8 cm; Auburn Plastics, Chicago, IL). Placement of tubing within the abomasum was confirmed every 10 d. Fat was infused three times daily (250 g at 0800, 1400, and 1900 h). Cows were turned out for 2 h of exercise following infusion of fat at 0800 h. Body weights were recorded twice weekly after the a.m. milking.

Sample Collection and Chemical Analyses

Feed samples were collected daily and composited each week for DM analysis. Chemical analyses of feed samples collected in the final 2 wk of each period were conducted at a commercial laboratory (Jefferson Laboratories, Jefferson, MD). An indigestible marker, ytterbium chloride (Research Chemicals, Phoenix, AZ), was added at 20 ppm to diet DM during the final 2 wk of each period so that DM and energy digestibility could be estimated. Equal percentages of orts collected during the last 5 d of experimental periods were composited and sampled. Equal volumes of fecal samples were collected from the rectum three times (0800, 1400, and 2000 h) daily between d 17 and 21 of each period and were kept at 4°C. At the end of collection periods, samples for each cow were mixed thoroughly, and subsamples of feed and feces were frozen at −20°C. Ytterbium content of feed and feces was quantitated by atomic absorption spectrophotometry (Model 5000; Perkin Elmer, Norwalk, CT) and gross energy by adiabatic bomb calorimetry (Parr Instruments, Moline, IL). Blood samples were collected from the coccygeal vein into 10-ml vacutainers (Becton Dickinson, Rutherford, NJ) just prior to each of the three fat infusions on d 16 and 17 of each experimental period.

Milk samples were collected at a.m. and p.m. milkings twice each week throughout the study and analyzed for SCC (Fossomatic 215 Cell Counter; Foss, Eden Prarie, MN), fat, lactose, and protein concentrations (Milkoscan 203B; Foss) at a commercial laboratory (Mid-East Milk Lab Services, Inc., Hagerstown, MD). Total solids content of milk was calculated as the sum of fat, protein, lactose, and mineral contents (a constant .7%). Two additional aliquots of milk were obtained from each cow at a.m. and p.m. milkings on 2 d during the final week of each period. One aliquot was frozen at −20°C for FA analysis and the second at −80°C for analysis of lipoprotein lipase (LPL; EC 3.1.1.34) activity.

The activity of LPL in milk was quantified as described by Hernell et al. (17) with modifications. Specifically, labeled TG emulsion was prepared by combining 6 μl of tri-[9,10-^{3}H]oleoylglycerol (185 kBq/ml; Amersham, Arlington Heights, IL), 46.5 μl of triolein (Sigma Chemical Co., St. Louis, MO), 1 ml of 10% (wt/vol) gum arabic (Sigma Chemical Co.), .48 ml of 1*M* Tris·HCl, pH 8.5, and .52 ml of cold distilled deionized water in a 12-ml plastic centrifuge tube on ice. This mixture was sonicated (setting 3; 50% pulse; Branson Sonifier 250, Danbury, CT) on ice for two 3-min periods with a 1-min rest period between. Milk samples were diluted 1:50 with barbital buffer (sodium barbital, 5 m*M*, pH 7.4; Sigma Chemical Co.). Incubations were for 15 min at 37°C in glass tubes; all tubes contained 50 μl of emulsion, 50 μl of heat-inactivated (60°C, 20 min) bovine serum, 32 μl of 18.7% (wt/vol) bovine serum albumin (Sigma Chemical Col.), 18 μl of distilled deionized water, and 50 μl of appropriately diluted sample, standard, or control. The LPL activity in samples was corrected for LPL activity in blanks containing heat-inactivated serum and no sample.

Adipose biopsies were taken from the rump of each cow on d 20 of each period. The area

to be sampled was cleaned and injected with 2% Lidocaine™ HCl (Phoenix Pharmaceutical, Inc., St. Joseph, MO). A 1-cm incision through skin and connective tissue allowed insertion of a soft tissue biopsy needle to a depth of approximately 1 cm (2.1 mm × 114 mm; Becton Dickinson). Tissue (80 to 120 mg) was removed and rinsed with .5 *M* KCl. Samples were stored in known volumes of .5 *M* KCl at –80°C preceding LPL and FA analysis.

Adipose tissue from the rumps of a steer, a Holstein cow in early lactation, and a nonlactating, nonpregnant Holstein cow was used in preliminary studies to optimize sample sizes, concentration of heparin needed to release LPL, incubation times, and volumes of heat-inactivated serum and eluate. Minced adipose tissue (20 to 50 mg; triplicate replication) was preincubated in 250 μl of Krebs-Ringer bicarbonate buffer with 5 IU of heparin (bovine lung; Sigma Chemical Co.) per tube for 30 min at 37°C in a shaking incubator to release LPL. An eluate volume of 50 μl subsequently was assayed for LPL activity as described for milk, but incubations were for 120 min instead of 15 min.

Representative samples for FA analysis (100 μl of plasma, infused fat, or milk; .5 g of feed; or 10 to 40 mg of adipose tissue) plus 100 μl of trinonadecenoin internal standard (2 mg/ml; NuChek Prep Inc., Elysian, MN) were combined in screw-capped pyrex tubes with 2 ml of methanolic·HCl (1.7*N*) and 1 ml of methylene chloride cosolvent, vortexed, and incubated overnight at 80°C. After cooling to 23°C, methyl esters of FA were first extracted with 2 ml of petroleum ether, .5 ml of distilled water, and 1 ml of saturated KCl and then extracted twice more with 2 ml of petroleum ether. Extracted FA methyl esters were dried at 37°C under a gentle stream of N, redissolved in .1 to .5 ml of isooctane, and transferred to GLC vials, which then were sealed. Fatty acids were quantified using a 30-m fused silica capillary column (.25 mm i.d.) coated with SP™-2380 (Supelco Inc., Bellefonte, PA) in a Hewlett-Packard Model 5890 GLC (Avondale, PA) equipped with a flame ionization detector.

Plasma NEFA concentrations were determined with a modified (22) NEFA-C procedure (WAKO Pure Chemicals Industries, Ltd., Osaka, Japan) and TG with the Triglyceride G kit (WAKO). Milk citrate concentration was determined enzymatically in all milk samples collected in the final week of each period (9). Insulin (4) and somatotropin (6) were assayed by double antibody radioimmunoassay. Intra- and interassay coefficients of variation were between 5 and 10% for all assays.

Calculations and Statistics

Average responses during the final experimental week of each period were analyzed by ANOVA for a Latin square design (30) using the general linear models procedure of SAS (28). Mean comparisons of interest were 1) uninfused control versus the mean of *cis* and *trans* treatments and 2) *cis* versus *trans* treatment.

RESULTS

Intake Response

One of 6 cows was infused with only 500 g/d for the *trans* treatment because she refused to eat when infused with any greater amount. Data from this cow were included in all statistical analyses.

Dry matter intake was lower for the *cis* and *trans* treatments compared with DMI of the uninfused control (Table 3). Amounts of $C_{16:0}$ and all C_{18} FA provided were greater for *cis* and *trans* treatments than for the uninfused control. The amount of *cis*-$C_{18:1}$ FA provided was greater for the *cis* than for the *trans* treatment (545 vs. 211 g/d), and the amount of *trans*-$C_{18:1}$ FA provided was greater for the *trans* than for the *cis* treatment (308 vs. 2 g/d). Although unintentional, the amount of $C_{18:2}$ FA provided was greater for the *cis* than for the *trans* treatment (184 vs. 171 g/d). Dry matter and energy digestibilities determined from fecal grab samples and Yb ratios were similar among treatments (Table 3). Calculated (23) NE_L intake was greater for *cis* and *trans* treatments than for the uninfused control. Amount of NE_L required, calculated using measured milk yield and BW values, was similar among treatments. Estimated energy balance was more positive for *cis* and *trans* treatments than for the uninfused control and was more positive for the *trans* than for the *cis* treatment. The amount of CP consumed was greater for the uninfused control than for *cis*

TABLE 3. Dry matter intake and related variables.[1]

	Treatment				Contrasts	
	Control	*cis*	*trans*	SE	Control vs. *cis* + *trans*	*cis* vs. *trans*
n	6	6	6		*P*	
DMI, kg/d	24.2	22.8	23.5	.3	.02	
Fatty acid (FA) intake, g/d						
$C_{16:0}$	55	145	150	4	.0001	
$C_{18:0}$	14	127	125	4	.0001	
cis-$C_{18:1}$	63	545	211	5	.0001	.0001
trans-$C_{18:1}$[2]	2	2	308	10	.0001	.0001
$C_{18:2}$	136	184	171	3	.0001	.01
Total	290	1022	984	26	.0001	
FA, % of DMI	1.2	4.5	4.2	.1	.0001	.09
Digestibility, %						
DM	65.7	63.3	66.3	2.1		
Energy	62.1	61.5	63.9	2.1		
BW, kg	607	601	604	4		
DMI/BW, %	3.98	3.81	3.88	.06	.09	
NE_L, Mcal/d						
Intake	40.6	42.6	43.5	.6	.006	
Required[3]	41.6	40.6	38.6	1.1		
Balance	−1.0	2.0	5.0	.9	.004	.05
CP (N × 6.25), kg/d						
Intake	5.7	5.4	5.6	.1	.03	
Required[3]	4.2	4.1	3.9	.1		
Excess	1.5	1.3	1.7	.1		.06

[1]One of 6 cows on the *trans* treatment received 500 g of fat/d, and the rest received 750 g of fat/d.

[2]Includes all *trans*-$C_{18:1}$ fatty acid isomers.

[3]Required NE_L and CP values were calculated using milk yield and BW responses measured for each treatment (23).

TABLE 4. Milk yield, composition, and lipoprotein lipase activity.

	Treatment				Contrasts	
	Control	*cis*	*trans*	SE	Control vs. *cis* + *trans*	*cis* vs. *trans*
n	6	6	6		*P*	
Milk, kg/d	47.0	46.3	47.0	1.1		
Fat, %	3.45	3.27	2.59	.21	.08	.05
Protein, %	3.08	2.97	3.06	.03		.08
Lactose, %	4.93	4.93	4.85	.03		.09
Total solids, %	12.16	11.85	11.15	.22	.04	.06
Yield in milk, g/d						
Fat	1605	1512	1211	93	.06	.05
Protein	1443	1372	1424	35		
Lactose	2298	2282	2273	57		
Total solids	5675	5490	5237	132	.09	
Citrate, mmol/L	7.83	8.68	9.99	.35	.007	.02
Lipoprotein lipase activity[1]	1.68	1.38	1.53	.07	.02	
SCC, × 1000	133	115	175	42		

[1]Units of lipoprotein lipase activity are micromoles of FFA released per min per milliliter of milk.

TABLE 5. Fatty acid composition of milk fat.

	Treatment				Contrasts	
	Control	*cis*	*trans*	SE	Control vs. *cis* + *trans*	*cis* vs. *trans*
n	6	6	6		*P*	
Fatty acid, g/100 g of fatty acid methyl esters						
$C_{10:0}$	1.5	1.1	.9	.1	.01	
$C_{12:0}$	4.1	3.0	2.5	.2	.0002	.08
$C_{14:0}$	13.4	10.5	10.2	.3	.0001	
$C_{16:0}$	35.0	25.6	23.9	.6	.0001	.09
$C_{18:0}$	10.2	11.2	10.6	.5		
$C_{14:1}$	1.0	.8	1.1	.1		.002
$C_{16:1}$	1.9	1.5	1.7	.1	.01	.06
cis-$C_{18:1}$	23.3	34.7	31.7	1.4	.0005	
trans-$C_{18:1}$[1]	1.9	3.1	8.0	.7	.002	.0009
$C_{18:2}$	3.8	4.7	5.4	.2	.004	.09

[1]Includes all *trans*-$C_{18:1}$ fatty acid isomers.

and *trans* treatments but exceeded calculated requirements for all treatments.

Milk Yield and Composition

Milk yield was similar among treatments (Table 4), but milk fat percentage and yield tended to be lower for *cis* and *trans* treatments than those for the uninfused control. Milk fat percentage and yield were lower for the *trans* than for the *cis* treatment. Lactose and total solids percentages tended to be lower for the *trans* than for the *cis* treatment, but yields in milk were similar.

Concentration of citrate in milk was higher for *cis* and *trans* treatments than for the uninfused control and was higher for the *trans* than for the *cis* treatment. Activity of LPL in milk was lower for *cis* and *trans* treatments than for the uninfused control. Milk SCC was similar among treatments.

Concentrations of $C_{10:0}$ to $C_{16:0}$ FA in milk were lower for *cis* and *trans* treatments than for the uninfused control (Table 5). Both *cis*-$C_{18:1}$ and *trans*-$C_{18:1}$ FA were present in greater concentrations for *cis* and *trans* treatments than for the uninfused control. However, the concentration of *trans*-$C_{18:1}$ FA in milk was greater for the *trans* than for the *cis* treatment (8 vs. 3.1 g/100 g of FA methyl esters).

Hormones, Metabolites, and FA in Blood

Concentrations of TG and NEFA in plasma were higher for the *cis* and *trans* treatments than for the uninfused control and were similar between *cis* and *trans* treatments (Table 6). Concentration of insulin in plasma tended to be higher, but concentration of somatotropin was lower for *cis* and *trans* treatments than for the uninfused control.

Concentration of $C_{16:0}$ FA in plasma was lower for the *trans* than for the *cis* treatment (Table 6). Relative concentrations of both *cis*- and *trans*-$C_{18:1}$ FA were higher for *cis* and *trans* treatments than for the uninfused control. In addition, concentration of *cis*-$C_{18:1}$ FA was higher for the *cis* than for the *trans* treatment, and concentration of *trans*-$C_{18:1}$ FA was higher for the *trans* than for the *cis* treatment. Concentration of $C_{18:2}$ FA in plasma was lower for the *cis* than for the *trans* treatment.

Activity of LPL in adipose tissue (mean across treatments ± SE = .14 ± .01 μmol of FFA released/h per g of tissue) and concentrations of FA in adipose tissue were similar among treatments ($C_{16:0}$ 27 ± 1; $C_{18:0}$ 19 ± 1; *cis*-$C_{18:1}$ 22 ± 4; *trans*-$C_{18:1}$ 2 ± .3; $C_{18:2}$ 13 ± 2 g of FA/100 g of FA methyl ester).

DISCUSSION

Supplemental dietary fat for dairy cattle depresses the synthesis of short- and medium-

TABLE 6. Concentrations of hormones, metabolites and fatty acids in blood.

	Treatment				Contrasts	
	Control	*cis*	*trans*	SE	Control vs. *cis* + *trans*	*cis* vs. *trans*
n	6	6	6		*P*	
Triglyerides,[1] mg/L	183	218	209	9	.02	
NEFA, μeq/L	159	204	189	9	.01	
Somatotropin, μg/L	3.69	2.85	1.94	.33	.01	.09
Insulin, mU/L	20.8	25.3	24.8	1.8	.09	
Fatty acids in plasma, g/100 g of fatty acid methyl esters						
$C_{14:0}$	.5	.5	.5	.03		
$C_{16:0}$	10.2	10.2	9.3	.2		.03
$C_{18:0}$	13.4	13.8	13.1	.4		
$C_{14:1}$	.3	.3	.3	.03	.07	
$C_{16:1}$	1.1	1.0	1.1	.06		
cis-$C_{18:1}$	6.3	11.4	8.7	.4	.0001	.002
trans-$C_{18:1}$[2]	.4	.7	2.6	.2	.001	.0002
$C_{18:2}$	48.5	45.6	48.4	.7		.01

[1]Includes all *trans*-$C_{18:1}$ fatty acid isomers.

chain length FA in mammary tissue and alters the proportions of specific FA present in milk fat (14, 32). Bacteria in the rumen biohydrogenate unsaturated FA to produce *trans*-$C_{18:1(n-7)}$ FA; chemical hydrogenation produces a combination of n-7, n-8, and n-9 *trans*-$C_{18:1}$ isomers. In the present study, abomasal infusion was used to avoid ruminal biohydrogenation of supplemental fat. Our goal was to increase the quantity of all positional isomers of *trans*-$C_{18:1}$ FA reaching the small intestine in order to evaluate the effects of monounsaturated *trans*-$C_{18:1}$ FA on milk fat synthesis and composition. Data suggest that reduced milk fat percentage and yield for the *trans* treatment may have resulted from the combined effects of reduced de novo FA synthesis and reduced formation of TG in the mammary gland.

Specifically, estimates of the apparent digestibility of long-chain FA are typically in excess of 65% and vary with chain length and degree of unsaturation (10, 14, 25). In addition, mammary extraction of circulating *trans*-$C_{18:1}$ FA was estimated to be 74% (34). However, in the present study, the apparent transfer of abomasally infused *trans*-$C_{18:1}$ FA into milk fat was 22% for the *trans* treatment (Tables 3, 4, and 5). Thus, the observation that acyl transferases in mammary tissue preferentially esterified *cis*- compared with *trans*-$C_{18:1}$ FA (2) supports the theory that TG synthesis in mammary tissue was reduced for *trans* treatment.

Dietary or abomasally infused fat reduces FA synthesis in mammary tissue (3, 12). Consequently, a reduction in the NADPH requirement for FA synthesis results in elevated milk citrate concentrations, reduced isocitrate dehydrogenase activity, and reduced tricarboxylic acid cycle activity in mammary tissue (5, 12, 13). In agreement with these observations, milk citrate concentrations were greater for *cis* and *trans* treatments than for the uninfused control. Further, the concentration of citrate in milk was greater for the *trans* than for the *cis* treatment, suggesting that, compared with *cis*-$C_{18:1}$ FA, *trans*-$C_{18:1}$ FA more effectively inhibit FA synthesis in mammary tissue.

The LPL associated with the capillary endothelium hydrolyzes FA of circulating TG, thereby making FA available for tissue removal. Long-chain FA ($C_{18:0}$ and longer) in bovine milk are derived exclusively from blood, presumably from the low and very low density lipoprotein fractions (11, 26, 31). In addition, the activity of LPL in milk may not reflect the activity in mammary tissue in all species. For example, in rats, LPL activity is high in mammary tissue but very slight in milk (15, 16). In contrast, in guinea pigs, the activity

of LPL in milk reflects that in mammary secretory tissue (21). Bovine milk contains a lipase with properties similar to tissue LPL (1, 20, 21). Thus, the measurement of LPL activity in milk may be used as a noninvasive means to evaluate relative effects of treatments on the activity of LPL in mammary tissue.

In the present study, the total concentration of $C_{18:1}$ FA in milk was greater for *cis* and *trans* treatments than for the uninfused control despite the lower activity of LPL measured in milk. Thus, the effects of *trans*-$C_{18:1}$ FA on milk fat percentage and yield are probably not mediated at the level of mammary LPL.

Comparison of the amounts of long-chain FA consumed with those secreted in milk each day suggested that mobilization of stored lipid to support milk fat secretion was greater for uninfused controls than for the *cis* and *trans* treatments (Tables 3, 4, and 5). In addition, a low activity of LPL in subcutaneous adipose tissue, combined with similar FA composition among treatments, suggested that adipose tissue FA deposition at the expense of milk fat synthesis either did not occur or was not detectable with the methods used. Thus, the fate of the majority of abomasally infused *trans*-$C_{18:1}$ FA could not be determined from these data. Because the rate of turnover of FA within fat depots varies, sampling of additional sites or the utilization of more specific techniques would strengthen conclusions regarding the fate of abomasally infused *trans*-$C_{18:1}$ FA.

Somatotropin and insulin influence the partitioning of nutrients during lactation (8, 27). Supplemental dietary fat induces lower somatotropin and higher insulin concentrations in serum (8, 27). However, in the present study, the decline in somatotropin and the increase in insulin concentration in serum were similar between *cis* and *trans* treatments, suggesting that these hormones did not mediate differences in the utilization of nutrients between these treatments.

Finally, stearoyl-coenzyme A desaturase-catalyzed conversion of blood-derived $C_{18:0}$ FA provides 30 to 40% of *cis*-$C_{18:1}$ FA in milk (19). Lack of a difference in milk *cis*-$C_{18:1}$ FA concentrations between *cis* and *trans* treatments argues against the hypothesis that *trans*-$C_{18:1}$ FA inhibit stearoyl-coenzyme A desaturase, thereby reducing milk fat percentage and yield. Thus, reduced milk fat percentage and yield for the *trans* compared with those for the *cis* treatment may have resulted from the combined effects of reduced de novo FA synthesis and reduced formation of TG in the mammary gland.

CONCLUSIONS

These data clearly demonstrated that abomasal infusion of *trans*-$C_{18:1}$ FA depressed milk fat percentage and yield more than *cis*-$C_{18:1}$ FA. We speculate that reduced synthesis of FA and reduced activity of acyl transferase in mammary tissue, and thus reduced TG synthesis, contributed to depressed milk fat percentage for the *trans* treatment.

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REFERENCES

1 Askew, E. W., R. S. Emery, and J. W. Thomas. 1970. Lipoprotein lipase of the bovine mammary gland. J. Dairy Sci. 53:1415.
2 Askew, E. W., R. S. Emery, and J. W. Thomas. 1971. Fatty acid specificity of glyceride synthesis by homogenates of bovine mammary tissue. Lipids 6:777.
3 Banks, W., J. L. Clapperton, and A. K. Girdler. 1990. Effect of dietary unsaturated fatty acids in various forms on the de novo synthesis of fatty acids in the bovine mammary gland. J. Dairy Res. 57:179.
4 Barnes, M. A., G. W. Kazmer, R. M. Akers, and R. E. Pearson. 1985. Influence of selection for milk yield on endogenous hormones and metabolites in Holstein heifers and cows. J. Anim. Sci. 60:271.
5 Bauman, D. E., and C. L. Davis. 1974. Biosynthesis of milk fat. Page 31 *in* Lactation: A Comprehensive Treatise. Vol II. B. L. Larson and V. R. Smith, ed. Academic Press. New York, NY.
6 Capuco, A. V., J. E. Keys, and J. J. Smith. 1989. Somatotropin increases thyroxine-5'-monodeiodinase activity in lactating mammary tissue of the cow. J. Endocrinol. 121:205.
7 Coppock, C. E., and D. L. Wilks. 1991. Supplemental fat in high-energy rations for lactating cows. Effects on intake, digestion, milk yield, and composition. J.

Anim. Sci. 69:3826.
8 Cummins, K. A., and J. L. Sartin. 1987. Response of insulin, glucagon, and growth hormone to intravenous glucose challenge in cows fed high fat diets. J. Dairy Sci. 70:277.
9 Dagley, S. 1974. Citrate UV spectrophotometric determination. Page 1562 *in* Methods of Enzymatic Analysis. H. U. Bergmeyer, ed. Academic Press, New York, NY.
10 Drackley, J. K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. J. Dairy Sci. 75:1517.
11 Emery, R. S. 1973. Biosynthesis of milk fat. J. Dairy Sci. 56:1187.
12 Faulkner, A., and J. L. Clapperton. 1981. Changes in the concentrations of some minor constituents of milk from cows fed low- or high-fat diets. Comp. Biochem. Physiol. 68A:281.
13 Faulkner, A., and H. T. Pollock. 1989. Changes in the concentration of metabolites in milk from cows fed diets supplemented with soybean oil or fatty acids. J. Dairy Res. 56:179.
14 Grummer, R. R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74:3244.
15 Hamosh, M., T. R. Clary, S. S. Chernick, and R. O. Scow. 1970. Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. Biochim. Biophys. Acta 210:473.
16 Hamosh, M., and R. O. Scow. 1971. Lipoprotein-lipase activity in guinea pig and rat milk. Biochim. Biophys. Acta 231:282.
17 Hernell, O., T. Egelrud, and T. Olivecrona. 1975. Serum stimulated lipases (lipoprotein lipases). Immunological crossreaction between the bovine and the human enzymes. Biochim. Biophys. Acta 381:233.
18 Katz, I., and M. Keeney. 1966. Characterization of the octadecenoic acids in rumen digesta and rumen bacteria. J. Dairy Sci. 49:962.
19 Kinsella, J. E. 1972. Stearyl CoA as a precursor of oleic acid and glycerolipids in mammary microsomes from lactating bovine: possible regulatory step in milk triglyceride synthesis. Lipids 7:349.
20 Korn, E. D. 1962. The lipoprotein lipase in cow's milk. J. Lipid Res. 3:246.
21 McBride, O. W., and E. D. Korn. 1963. The lipoprotein lipase of mammary gland and the correlation of its activity to lactation. J. Lipid Res. 4:17.
22 McCutcheon, S. N., and D. E. Bauman. 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin-releasing hormone in lactating cows. J. Dairy Sci. 69:44.
23 National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, DC.
24 Okuyama, H., W.E.M. Lands, F. D. Gunstone, and J. A. Barve. 1972. Selective transfers of *trans*-ethylenic acids by acyl coenzyme A: phospholipid acyltransferases. Biochemistry 11:4392.
25 Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753.
26 Palmquist, D. L., and W. Mattos. 1978. Turnover of lipoproteins and transfer to milk fat of dietary (1-carbon-14) linoleic acid in lactating cows. J. Dairy Sci. 61:561.
27 Palmquist, D. L., and W. Moser. 1981. Dietary fat effects on blood insulin, glucose utilization, and milk protein content of lactating cows. J. Dairy Sci. 64: 1664.
28 SAS® User's Guide: Statistics, Version 5 Edition. 1985. SAS Inst., Inc., Cary, NC.
29 Selner, D. R., and L. H. Schultz. 1980. Effect of feeding oleic acid or hydrogenated vegetable oils to lactating cows. J. Dairy Sci. 63:1235.
30 Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Co., San Francisco, CA.
31 Storry, J. E. 1970. Ruminant metabolism in relation to the synthesis and secretion of milk fat. J. Dairy Res. 37:139.
32 Sutton, J. D. 1989. Altering milk composition by feeding. J. Dairy Sci. 72:2801.
33 Teter, B. B., J. Sampugna, and M. Kenney. 1990. Milk-fat depression in C57B1/6J mice consuming partially hydrogenated fat. J. Nutr. 120:818.
34 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.
35 Timmen, H., and S. Patton. 1988. Milk fat globules: fatty acid composition, size and in vivo regulation of fat liquidity. Lipids 23:685.