

Effects of Fat Source and Copper on Unsaturation of Blood and Milk Triacylglycerol Fatty Acids in Holstein and Jersey Cows¹

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ABSTRACT

Fatty acid composition of plasma triacylglycerides and milk fat was analyzed from Holstein and Jersey cows with control or depleted copper status and fed roasted whole soybeans or tallow. Conjugated linoleic acid in plasma was higher in Jersey cows. Dietary fat source influenced the proportions of all fatty acids in plasma and in milk, except for conjugated linoleic acid in milk. Feeding soybeans increased plasma C_{14:1}, C_{18:0}, C_{18:2}, and conjugated linoleic acid, and decreased C_{14:0}, C_{16:0}, C_{16:1}, and *cis*- and *trans*-C_{18:1} compared with feeding tallow. Low copper diets decreased C_{18:0} and increased *cis*- and *trans*-C_{18:1}, and conjugated linoleic acid in plasma. A fat source × copper status interaction occurred for *cis*-C_{18:1} in plasma. Proportions of C_{4:0} to C_{14:0} were higher, and *cis*-C_{16:1}, *cis*- and *trans*-C_{18:1}, and conjugated linoleic acid were lower in milk fat of Jersey compared with Holstein cows. Generally, the effects of copper depletion were less apparent in milk than in plasma. Copper depletion increased C_{4:0}, *trans*-C_{18:1}, and conjugated linoleic acid, and decreased C_{16:1} in milk. Feeding whole soybeans increased C_{4:0} to C_{14:0}, C_{18:0}, C_{18:2}, and C_{18:3}, and decreased C_{14:1}, C_{16:0}, C_{16:1}, and *cis*- and *trans*-C_{18:1} in milk. Breed × fat interactions occurred for C_{4:0}, C_{14:1}, C_{16:1}, and conjugated linoleic acid in milk. Copper status × fat source interaction occurred for *trans*-C_{18:1}. The breed × copper status interaction was apparent in milk fat for C_{16:1} and C_{18:0} and conjugated linoleic acid in milk. Both C_{18:0} and *trans*-C_{18:1} were desaturated by mammary tissue; however, whereas desaturation of C_{18:0} was linear, desaturation of *trans*-C_{18:1} reached a plateau that could have been caused by presence of the *trans*-10 isomer, which is not

desaturated and was not separated from *trans*-11 C_{18:1} in our analysis. Comparison of the plasma triacylglycerol fatty acid profile with the milk fat profile was useful to interpret separate events of biohydrogenation in the rumen and desaturation by the mammary gland.

(**Key words:** breeds, fat, copper, Δ -9 desaturase)

Abbreviation key: CLA = conjugated linoleic acid, TAG = triacylglycerol, TVA = *trans*-vaccenic acid.

INTRODUCTION

The functional and nutritional properties of milk fat are strongly influenced by the array of fatty acids incorporated into its structure (16). The degree of unsaturation can be modified to influence such factors as softness of butter (2), susceptibility to development of spontaneous oxidized flavor (7, 15, 17), and nutritional value (16, 29). Because of ruminal biohydrogenation, manipulation of unsaturation of milk fat by dietary means is limited (19, 28, 41). An important compensation for biohydrogenation is the activity of Δ -9 desaturase (stearyl-CoA desaturase; E.C. 1:14.99.5). This enzyme is active in the intestinal mucosa (4), adipose tissue (34), and mammary gland (5, 20) of lactating cows but is not active in liver (34).

Many types of supplemental fats have been fed to lactating cows as an energy source or to modify milk fat composition (19, 28). Under certain ruminal conditions, the biohydrogenation intermediates, *cis*-9, *trans*-11 octadecadienoic acid (rumenic acid, generically known as conjugated linoleic acid, or CLA) and *trans*-11 octadecenoic acid (*trans*-vaccenic acid, or TVA) accumulate in the rumen because of incomplete biohydrogenation. *Trans*-vaccenic acid usually occurs in milk fat at three to five times the concentration of CLA (30). These intermediates are of interest and concern: CLA has been shown to be a potent anticarcinogenic agent (39) and *trans* monoenes have been implicated as the cause of milk fat depression (40).

Many *trans* monoenes are substrates for Δ -9 desaturase, forming *cis*-9, *trans*-x dienes (21). Conversion of TVA to CLA has been estimated in lactating cows (9) and quantified in mice, where 50% of TVA stored in the body was desaturated to CLA (32). Activity of Δ -9

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desaturase may differ among breeds of cows (3, 12). Copper has been reported to influence activity of Δ -9 desaturase (36). Further, copper is absorbed more efficiently and is found in higher concentrations in tissues of Jersey than Holstein cows (13). Copper also is a prooxidant and may influence oxidative stability of unsaturated milk lipids (31). Therefore, our objective was to investigate relationships of *cis*-C_{18:1} to C_{18:0}, and CLA to *trans*-C_{18:1} in plasma triacylglycerides (TAG) and milk fat of Jersey and Holstein cows fed diets containing tallow or roasted whole soybeans under conditions of adequate or depleted copper status.

MATERIALS AND METHODS

Animals

Twelve Jersey and 12 Holstein cows, 60 to 200 DIM, were divided into three blocks and assigned randomly to four treatments arranged factorially: control or depleted copper status, and tallow (3% of diet DM) or commercially roasted whole soybeans (15% of diet DM). Cows were adjusted to control or depleted copper status during a 2-mo preliminary feeding period. Copper depletion was induced by supplementing molybdenum and additional sulfur in the diet. Status was verified by liver biopsy according to procedures approved by Ohio State University College of Agriculture Livestock Animal Use Committee. Details of diet composition and animal management are published elsewhere (23).

One-half of the cows in each breed and copper status were fed each fat supplement for 5 wk, then fat supplements were reversed. At the end of the wk 4 of each period, a mixed arterial-venous blood sample was obtained from the tail vein of each cow after the p.m. milking; milk samples were obtained from the following a.m. milking to compare specifically the fatty acid profiles of blood and milk triglycerides. Samples were not obtained from two Holstein cows in the second period.

Analyses

Blood samples were obtained in heparinized vacutainers (Becton Dickinson, Franklin Lakes, NJ), maintained on ice, and centrifuged to obtain plasma, which was frozen until processing for lipid separation. To obtain lipids, 6 ml of di-isopropyl ether:butanol (60:40, vol:vol) was added to 3 ml of plasma; the sample was vortexed and placed in a rotary shaker at 180 rpm for 30 min at room temperature (-20°C). Samples were centrifuged to separate the phases and the supernatant was transferred to a test tube, followed by evaporation of the solvent under N₂ in a dry bath at 40°C. When dry, 0.5 ml of hexane was added to each sample and tubes were capped. Lipid fractions were separated on

a 500-mg silicic acid column (3009250, Alltech Assoc., Deerfield, IL) according to Christie (11). The identity of fractions was verified against known standards by spotting aliquots of extracts on 20- × 20-cm silica gel TLC plates (Whatman, Inc., Clifton, NJ), which were developed with petroleum ether: diethyl ether: acetic acid (90:10:1, vol:vol:vol). Solvent was evaporated under N₂ from the TAG fraction, which was methylated by alkaline trans-esterification (10). Methyl esters were concentrated and transferred to vials for analysis by GLC. Methyl esters of milk fatty acids were prepared (35) and separated on a 30-m × 0.32-mm fused silica column coated with SP2340 (Supelco Inc., Belfonte, PA), using a Hewlett-Packard 5890 gas chromatograph with automated injection and data reduction (HP3365 Chemstation software, Hewlett-Packard Co., Santa Clarita, CA).

Statistical Analyses

The experimental design used to analyze plasma and milk fatty acid data (data collected during wk 4 and 9) was a split plot with the split plot treatments in a Latin square (8). Main plot effects were breed and copper, and fat source was the split plot treatment. The general linear models procedure of SAS (33) was used to analyze the data. The statistical model was $Y = \mu + R + \text{Row} + C + B + B*C + R*C*B + F + C*F + B*F + B*C*F + \text{error}$, where Y = dependent variable, R = replicate (i.e., block; 2 df), Row = effect of row (2 periods × 3 replicates = 5 df), C = effect of copper treatment (1 df), B = effect of breed (1 df), B*C = interaction between breed and copper (1 df), R*C*B = error term (6 df) to test B, C, and B*C, F = effect of fat treatment (1 df), and C*F (1 df), B*F (1 df), and B*C*F (1 df) = interactions between copper, breed, and fat, and error = error term (27 df) to test F, F*B, F*C, and F*B*C. The relationship between the ratio of *cis* C_{18:1}/(C_{18:0} + *cis*-C_{18:1}) in milk and plasma was quantified by linear regression (33). The relationship between the CLA/(*trans* C_{18:1} + CLA) ratio in milk and plasma was not linear. A segmented model with an initial quadratic phase followed by a plateau was derived using PROC NLIN (33). Significance is declared at $P < 0.05$ unless indicated otherwise.

RESULTS AND DISCUSSION

The fatty acid composition of diets and liver copper status of the cows are reported elsewhere (23).

Plasma TAG Fatty Acids

The profile of fatty acids of plasma TAG is shown in Table 1. Because Δ -9 desaturase activity of ruminant

Table 1. Plasma triacylglycerol fatty acid profiles of Holstein and Jersey cows fed roasted whole soybeans or tallow with control or low copper diets (least square means).

Fatty acid	Jersey				Holstein				SE ¹	SE ²	Effect ³
	Tallow		Soybean		Tallow		Soybean				
	Control	Low	Control	Low	Control	Low	Control	Low			
	g/100 g fatty acids										
C _{14:0}	2.40	2.97	2.06	1.83	3.13	2.67	2.31	1.56	0.38	0.25	F
C _{14:1}	0.65	0.66	0.97	0.75	0.58	0.50	0.76	1.21	0.13	0.18	F
C _{16:0}	28.31	27.66	20.34	19.64	29.15	29.15	20.36	20.00	1.23	0.51	F
C _{16:1}	1.60	1.93	1.09	1.59	2.00	1.91	1.37	1.56	0.29	0.27	F
C _{18:0}	40.16	29.33	45.78	43.73	34.64	33.76	43.89	46.13	2.70	2.40	C, F
<i>trans</i> -C _{18:1}	7.82	12.76	5.29	6.70	9.65	10.50	7.89	6.67	0.83	0.90	C × F (0.10)
<i>cis</i> -C _{18:1}	11.38	15.40	10.92	11.81	12.12	14.60	10.30	10.92	1.33	0.96	C × F (0.09)
C _{18:2}	2.30	3.31	7.95	7.05	3.10	2.75	6.57	6.13	0.70	0.86	C (0.08), F
CLA ⁴	0.07	0.69	0.96	1.43	0.12	0.07	0.72	0.92	0.18	0.23	C × F
Other	5.31	5.29	4.64	5.47	5.51	4.10	5.83	4.90	B, C, F
<i>cis</i> -C _{18:1} /C _{18:0}	0.29	0.55	0.27	0.27	0.36	0.48	0.24	0.20	0.05	0.06	B × C (0.06)
CLA/ <i>trans</i> -C _{18:1}	0.01	0.06	0.16	0.28	0.02	0	0.10	0.14	0.04	0.06	F

¹SE to test effects of breed, copper, and breed × copper.

²SE to test effects of fat and fat interactions.

³Effects: B = breed, C = copper, F = fat ($P < 0.05$, except as noted).

⁴CLA: conjugated linoleic acid.

intestinal mucosa is relatively low (4, 6), the pattern of unsaturated fatty acids in plasma TAG may provide insight into ruminal biohydrogenation activity. Breed effects were observed only for proportions of CLA ($P < 0.05$) and for the ratio of CLA to *trans*-C_{18:1} ($P < 0.10$). Concentrations of CLA were higher in Jersey cows and there was an interaction ($P < 0.06$) of breed with copper status, in that low copper status increased CLA in plasma TAG more in Jersey than in Holstein cows. The ratio of CLA to *trans*-C_{18:1} in plasma TAG was higher ($P < 0.10$) in Jersey cows than in Holstein cows. Proportions of C_{14:0}, C_{16:0}, C_{16:1}, *cis* C_{18:1}, and *trans* C_{18:1} were increased by feeding tallow, consistent with their higher content in tallow diets (23). Interestingly, C_{18:0} was higher in plasma TAG when soybeans were fed, suggesting more complete ruminal biohydrogenation of fatty acids released from soybeans. Though lower *trans*-C_{18:1} also may suggest more complete biohydrogenation of soybean unesterified fatty acids in the rumen; this must be interpreted with caution, because biohydrogenation of the *trans* 18:1 in the tallow is a rate-limiting process (26). Feeding soybeans increased C_{14:1}, C_{18:2}, and CLA in plasma TAG; the higher C_{18:2} suggests that a part of the soybeans escaped the rumen before complete release and biohydrogenation of the fatty acids. Lower C_{18:0} ($P < 0.10$) and increased *cis*-C_{18:1} ($P < 0.08$),

trans-C_{18:1} and CLA when low copper diets were fed suggest that biohydrogenation was less complete with this main effect. The interaction of copper and fat source ($P < 0.05$ for *cis*-C_{18:1}, $P < 0.10$ for C_{18:0} and *trans*-C_{18:1}) suggests that the depleted copper effect was greater when tallow was fed and that the effect may have been specifically on biohydrogenation of C_{18:1} isomers to C_{18:0}. The ratio of *cis*-C_{18:1} to C_{18:0} was increased by feeding tallow and the effect of copper depletion was greater when tallow was fed. The ratio of CLA to *trans*-C_{18:1} also was increased by copper depletion ($P < 0.10$) but decreased by feeding tallow.

Milk Fatty Acids

Milk fatty acid profiles are shown in Table 2. Values were similar to those reported in Morales et al. (23) but were a separate data set to compare with plasma values reported here. Breed × copper interactions were observed for C_{16:1}, C_{18:0}, and CLA. Proportions of C_{4:0} to C_{14:0} and C_{18:0} were higher, and C_{16:1}, *cis*-C_{18:1}, *trans*-C_{18:1}, and CLA were lower in Jersey compared with Holstein cows. Similar differences were reported previously (3). Copper depletion increased C_{4:0}, *trans*-C_{18:1}, and CLA in milk fat, but generally, effects of copper depletion were less apparent than in plasma. Feeding

roasted whole soybeans resulted in higher proportions of C_{4:0} to C_{14:0}, C_{18:0}, C_{18:2}, and C_{18:3}, and lower C_{14:1}, C_{16:0}, C_{16:1}, *cis*-C_{18:1}, and *trans*-C_{18:1} in milk fat. The ratio of *cis*-C_{18:1} to C_{18:0} was higher in Holstein cows and when tallow was fed. Breed × fat source interaction indicated that this ratio was changed more in Holstein than in Jersey cows when dietary fat source was changed. Copper depletion increased this ratio in Jersey cows, but decreased it in Holstein cows. Feeding soybeans increased the ratio of CLA to *trans*-C_{18:1}.

A common characteristic of milk fat from Jersey cows and for feeding soybeans was a higher content of C_{4:0} to C_{14:0} fatty acids. Melting point of milk fat is lowered both by increased short and medium chain fatty acids and by increased ratio of C_{18:1} to C_{18:0}. The inverse relationship between proportion of C_{4:0} to C_{14:0} fatty acids and the ratio of C_{18:1} to C_{18:0} observed in this study may indicate that desaturation of C_{18:0} is responsive to the amount of short chain fatty acids present in the mammary tissue.

Fatty Acid Ratios

To examine the desaturase activity of the mammary gland in more detail, we calculated normalized ratios of *cis*-C_{18:1} to (C_{18:0} + *cis*-C_{18:1}) (hereafter referred to as 18:1 ratio) and CLA to (*trans*-C_{18:1} + CLA) (CLA ratio) in plasma TAG and milk (Table 3). This approach was taken because we did not measure absolute amounts of plasma TAG, because the greater number of individual fatty acids in milk fat influence their proportions compared with plasma, and because C_{18:0} can be desaturated to *cis*-C_{18:1}, and *trans*-C_{18:1} can be desaturated to CLA. Feeding tallow increased the 18:1 ratio in plasma and milk; this ratio also was increased in plasma by copper depletion and in milk of Holstein cows. The CLA ratio was lower in the plasma of Holstein than Jersey cows. The CLA ratio was lower in plasma and milk when tallow was fed and was increased in plasma and milk when low copper diets were fed (both *P* < 0.10). The effect of copper was greater in Holstein than in

Table 2. Milk fatty acid profile of Holstein and Jersey cows fed roasted whole soybeans or tallow with control or low copper diets. Treatment effects.

Fatty acid	Jersey				Holstein				SE ¹	SE ²	Effect ³
	Tallow		Soybean		Tallow		Soybean				
	Control	Low	Control	Low	Control	Low	Control	Low			
	g/100 g fatty acids										
C _{4:0}	3.79	4.06	4.03	4.32	2.65	3.56	3.65	4.26	0.28	0.08	B (<i>P</i> < 0.10) C, F, B × F
C _{6:0}	2.38	2.40	3.02	3.06	1.59	1.82	2.34	2.69	0.16	0.04	B, F
C _{8:0}	1.28	1.30	1.77	1.80	0.83	0.87	1.31	1.47	0.06	0.02	B, F
C _{10:0}	2.60	2.72	3.62	3.76	1.75	1.65	2.77	2.87	0.28	0.12	B, F
C _{12:0}	2.89	3.02	3.74	3.88	2.23	1.91	3.03	2.88	0.32	0.13	B, F
C _{14:0}	10.64	10.40	10.96	10.76	9.75	8.57	10.70	9.28	0.89	0.22	B (<i>P</i> < 0.10) F
C _{14:1}	1.24	1.13	0.93	0.94	1.73	0.94	1.05	0.46	0.11	0.02	F, B × F
C _{16:0}	31.40	29.92	26.66	26.41	31.68	30.24	25.44	25.77	4.60	1.35	F
C _{16:1}	1.68	1.72	0.94	1.09	3.08	2.40	1.33	0.83	0.07	0.07	B, C, F B × C, B × F
C _{18:0}	12.26	10.60	15.42	13.35	9.40	11.99	12.79	16.26	2.90	1.56	F, B × C
<i>trans</i> -C _{18:1}	4.04	6.56	2.21	3.10	4.96	5.64	3.95	3.20	0.83	0.62	B (<i>P</i> < 0.10) C (<i>P</i> < 0.10) F, C × F
<i>cis</i> -C _{18:1}	20.45	19.82	18.35	18.37	23.73	24.01	21.88	20.39	2.38	1.21	B, F
C _{18:2}	1.76	2.30	4.71	5.34	2.36	2.30	5.27	5.32	0.68	0.15	F
C _{18:3}	0.35	0.42	0.94	0.99	0.43	0.45	1.00	1.01	0.02	0.01	F
CLA ⁴	0.61	1.04	0.63	0.84	0.81	0.89	1.03	0.94	0.02	0.02	B, C, B × C, B × F (<i>P</i> < 0.10)
Other	2.63	2.59	2.07	1.99	3.02	2.76	2.46	2.37
<i>cis</i> -C _{18:1} /C _{18:0}	1.68	1.88	1.19	1.42	2.56	2.24	1.73	1.06	0.04	0.04	B, F B × C, B × F
CLA/ <i>trans</i> -C _{18:1}	0.18	0.17	0.30	0.28	0.17	0.18	0.27	0.29	0.003	0.002	F

¹SE to test effects of breed, copper, and breed × copper.

²SE to test effects of fat and fat interactions.

³Effects: B = breed, C = copper, F = fat (*P* < 0.05, except as noted).

⁴CLA: conjugated linoleic acid.

Table 3. Ratios of *cis*-C_{18:1}/(C_{18:0} + *cis*-C_{18:1}) and CLA/(*trans*-C_{18:1} + CLA) in plasma triacylglycerol and milk of Holstein and Jersey cows fed roasted whole soybeans with control or lower copper diets.

Ratio	Jersey				Holstein				SE ¹	SE ²	Effect ³
	Tallow		Soybean		Tallow		Soybean				
	Control	Low	Control	Low	Control	Low	Control	Low			
<i>cis</i> -C _{18:1} /(C _{18:0} + <i>cis</i> -C _{18:1})											
Plasma	0.21	0.32	0.16	0.25	0.26	0.36	0.18	0.23	0.001	0.001	C, F
Milk	0.64	0.64	0.54	0.56	0.72	0.72	0.64	0.65	0.002	0.001	B, F
Milk/Plasma ⁴	3.04	2.06	3.32	2.32	2.91	2.11	3.52	2.92	0.08	0.10	C, F
CLA ⁵ /(<i>trans</i> -C _{18:1} + CLA)											
Plasma	0.020	0.028	0.12	0.18	0.019	0.014	0.082	0.11	0.001	0.001	B, F, C (<i>P</i> < 0.10)
Milk	0.15	0.14	0.22	0.21	0.14	0.20	0.21	0.26	0.001	0.001	C (<i>P</i> < 0.10) F, B × C
Milk/Plasma ⁶	6.73	4.65	1.89	1.41	10.56	8.23	3.93	2.67	2.29	4.63	B, C, F

¹SE to test effects of breed, copper, and breed × copper.

²SE to test effects of fat and fat interactions.

³B = Breed, C = copper, F = fat (*P* < 0.05, except as noted).

⁴Normalized 18:1 ratio in milk divided by normalized 18:1 ratio in plasma.

⁵CLA: conjugated linoleic acid.

⁶Normalized CLA ratio in milk divided by normalized CLA ratio in plasma.

Jersey cows, causing a significant breed × copper interaction. In all cases, both 18:1 and CLA ratios were higher in milk than in plasma, consistent with desaturation of C_{18:0} and *trans*-C_{18:1} by mammary Δ-9 desaturase and greater desaturase activity in mammary tissue than in the intestine (4, 6). The milk to plasma 18:1 ratio was higher for control copper and soybean diets, suggesting higher mammary desaturation of C_{18:0} when these diets were fed. The milk to plasma CLA ratio was higher in Holstein than Jersey cows, for control diets than low copper diets, and for tallow diets than soybean diets.

When all data were combined, the relationship between the 18:1 ratio in milk versus that in plasma was linear: Milk ratio = 0.527 + 0.448 (plasma ratio), *r*² = 0.28, *P* < 0.002, *n* = 46, indicating that 44.8% of 18:0 taken up was desaturated, and that mammary desaturation did not decrease as plasma unsaturation increased. Enjalbert et al. (14) reported that mammary desaturation of C_{18:0} was 52% over a range of mammary C_{18:0} uptake of 10 to 40 mmole/L of milk; this would tend to narrow the 18:1 ratio as C_{18:0} uptake increases. Doubling C_{18:1} uptake did not inhibit desaturase activity (14). Regression analysis of the CLA ratio of milk versus plasma revealed a more complex relationship. Although no treatment effects were significant, it is apparent that two populations of data exist (Figure 2). The plot of milk to plasma ratios for tallow diets (open circles) occurs over a narrower range of plasma ratio, and is steeper than the plot for soybean diets. Taken collectively, the relationship is quadratic (*P* < 0.002, *r*²

= 0.34), and milk CLA ratio reached a plateau at 0.23, when the plasma CLA ratio was near 0.15. Although the cause for different desaturation of C_{18:0} and *trans*-C_{18:1} is uncertain, there may have been a considerable amount of *trans*-10 monoene present, considering the low milk fat percentage of these cows (18). Our fatty acid analysis system did not separate the various isomers of *trans*-C_{18:1}. Because the *trans*-10 isomer is desaturated only to a limited extent (21); presence of

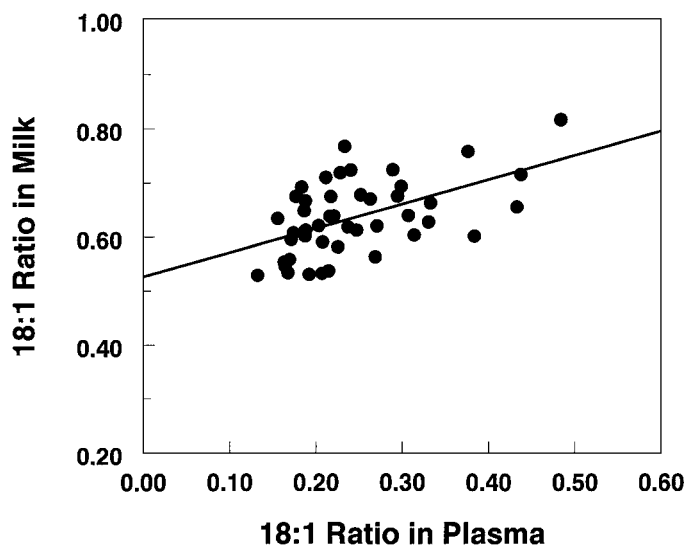


Figure 1. Ratio of *cis*-C_{18:1}/(C_{18:0} + *cis*-C_{18:1}) in milk vs *cis*-C_{18:1}/(C_{18:0} + *cis*-C_{18:1}) in plasma. 18:1 ratio in milk = 0.527 + 0.448 (plasma ratio), *r*² = 0.28, *P* < 0.002, *n* = 46.

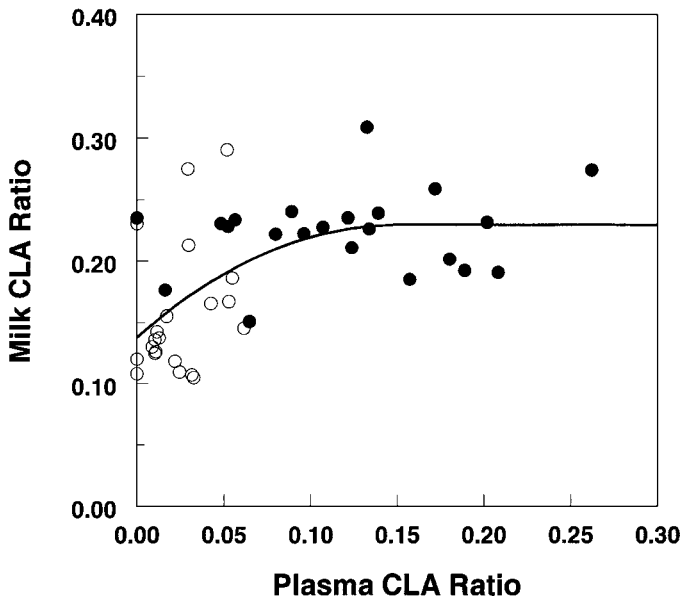


Figure 2. Ratio of conjugated linoleic acid (CLA)/(*trans*18:1 + CLA) in milk versus CLA/(*trans*18:1 + CLA) in plasma. CLA ratio in milk = $(0.138 \pm 0.0146) + (1.24 \pm 0.546)$ (CLA ratio in plasma) - (4.20 ± 3.48) (CLA ratio in plasma)², n = 45. Open circles = tallow, closed circles = whole soybeans.

trans-10 C_{18:1} in our samples could cause a curvilinear CLA ratio as *trans*-11 C_{18:1} became limiting. Although higher *trans*-10 C_{18:1} is possible for the soybean diet, it seems unlikely given the overall lower *trans*-C_{18:1} in milk when this diet was fed, and that the proportion of fatty acids synthesized de novo was higher when soybeans were fed. Despite uncertainty of the cause, we present the data to show that desaturation of *trans*-C_{18:1} isomer(s) by the mammary gland increases milk CLA content.

Ruminal Biohydrogenation

The lower ratio of *cis*-C_{18:1} to C_{18:0} and the lower proportion of *trans*-C_{18:1} in plasma suggest that ruminal biohydrogenation was more extensive when soybeans were fed, compared with tallow. This could occur if the oil were released slowly from the oilseed. Roasting of the soybeans presumably would cause slow degradation of the protein in membranes surrounding the fat, thus assuring slow release. However, C_{18:2} and CLA, both intermediates in biohydrogenation, were higher in plasma TAG when soybeans were fed. Higher C_{18:2} and C_{18:3} in plasma and milk caused by feeding soybeans would suggest that some of the soybean fatty acids were protected from ruminal biohydrogenation. The increased amount of C_{18:2} in milk of cows fed soybeans compared with those fed tallow was only 20 to 30 g/d (136% increase in C_{18:2} as a proportion of total milk

fatty acids). Assuming that 50% of intestinal fatty acids are secreted directly in milk (1, 27), the amount of C_{18:2} escaping biohydrogenation would need to be <100 g/d. We estimated ruminal biohydrogenation of C_{18:2} in soybeans to be >80% (data not shown), similar to the extent reported for other unsaturated fat sources (25, 41). Thus the higher C_{18:2} reported in milk fat when cows are fed whole soybeans (22, 24, 37) would appear to be caused by slow release of the large amount of C_{18:2} provided; the extent of biohydrogenation of unsaturated fatty acids from roasted whole soybeans was not compromised by the large amount of fat provided, so that the proportion of *trans*-C_{18:1} found in milk fat was actually lower than when tallow was fed. This speculation does not explain the higher proportion of CLA found in plasma when roasted whole soybeans were fed.

Ruminal biohydrogenation was less complete when cows were depleted of copper, as indicated by higher levels of all unsaturated fatty acids in plasma TAG. Whether this was caused directly by copper depletion, or by toxicity of the added molybdenum or changes in ruminal sulfur status is uncertain.

CONCLUSIONS

Dietary conditions causing copper depletion (low diet copper with added molybdenum and higher sulfur) decreased ruminal biohydrogenation, and the effect was greater when tallow was fed, compared with roasted whole soybeans. Although biohydrogenation of roasted whole soybeans is very efficient, with very little accumulation of partially hydrogenated intermediates, feeding soybeans increased C_{18:2} and C_{18:3} in milk, which increases potential for development of spontaneous oxidized flavor (38). Comparison of the composition of plasma TAG with that of milk fat is a useful indicator of ruminal biohydrogenation and mammary desaturation.

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