

# Milk Fat Composition of Holstein and Jersey Cows with Control or Depleted Copper Status and Fed Whole Soybeans or Tallow<sup>1</sup>

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## ABSTRACT

We studied effects of breed, dietary fat source, and dietary copper intake as factors known to influence unsaturation of milk fat and its potential for development of spontaneous oxidized flavor in milk. Twelve Holstein and 12 Jersey cows were allotted to three blocks with four cows of each breed. Cows within breed were allotted randomly within blocks and fed control or copper-depleting diets for 2 mo to achieve stable or depleted liver copper stores. Cows then were fed tallow or roasted whole soybeans in a two-period switchback (5 wk per period); during the last week of each period additional vitamin E (2000 IU/d) was added. Copper depletion for 2 mo decreased concentrations of copper in liver. Feed intake and milk yield were influenced only by breed. The proportions of C<sub>4:0</sub> to C<sub>14:0</sub> and C<sub>18:0</sub> in milk fat were higher, whereas C<sub>16:1</sub> and *cis*-C<sub>18:1</sub> were lower in Jersey cows. Feeding soybeans increased C<sub>4:0</sub> to C<sub>14:0</sub>, C<sub>18:0</sub>, C<sub>18:2</sub>, and C<sub>18:3</sub> in milk, and decreased C<sub>14:1</sub>, C<sub>16:0</sub>, C<sub>16:1</sub>, *trans*-C<sub>18:1</sub>, and *cis*-C<sub>18:1</sub>. Depleted copper status increased conjugated linoleic acid in milk. Several breed × fat source interactions for individual milk fatty acids occurred. Feeding soybeans decreased plasma concentrations of copper and zinc, and increased concentrations of α-tocopherol in plasma and milk. The concentration of zinc was higher in milk of Jersey cows. Depleted copper status tended to increase copper concentration in plasma and decreased copper in milk. Fat source did not influence plasma copper concentration when status was adequate, but plasma copper concentration was higher when tallow was fed to cows with depleted copper status. Supplementing vitamin E increased concentration of α-tocopherol in plasma and

milk and decreased concentration of zinc in milk. Factors influencing the potential for oxidized flavor development in milk can be manipulated by changing the diet of the cow.

**(Key words:** milk fat composition, soybeans, copper status, breeds)

**Abbreviation key:** CLA = conjugated linoleic acid, SOF = spontaneous oxidized flavor.

## INTRODUCTION

Changes in milk fat composition by feeding fat have been well documented (26). Most fat supplements increase the 18-carbon fatty acids of milk fat and increase unsaturation by increasing the proportion of C<sub>18:1</sub> in the milk fat. This is accomplished by coordination of biohydrogenation events in the rumen that convert dietary unsaturated oils primarily to C<sub>18:0</sub> and the desaturating activity of stearoyl-CoA desaturase in the intestinal mucosa and mammary gland (5). Increasing C<sub>18:2</sub> and C<sub>18:3</sub> in milk fat is more difficult because it requires that these dietary unsaturated fatty acids be protected from ruminal biohydrogenation (26). However, significant increases in C<sub>18:2</sub> and C<sub>18:3</sub> were observed when soybeans were fed (20, 34).

In recent years, dairy processors in Ohio have reported increased incidence of spontaneous oxidized flavor (SOF) during the winter feeding period. These occurrences seem to be related to feeding whole soybeans (25). In controlled studies (7), feeding extruded soybeans compared with feeding a calcium salt of palm oil increased concentrations of C<sub>18:2</sub> and C<sub>18:3</sub> in milk and SOF that was not reversed by feeding high amounts of vitamin E.

Copper in milk is a prooxidant (29) that may interact with increased unsaturation of milk fat to accelerate development of SOF; copper also has been reported to influence activity of stearoyl-CoA desaturase (33). Jersey cattle absorb copper more efficiently than Holsteins (12), and the activity of stearoyl-CoA desaturase in Jersey cows may be lower than in Holstein cows (4, 11); thus, the effect of breed on the interaction of dietary copper with dietary fat source was of interest.

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We undertook a study with the following objective: to compare the effects of feeding tallow or roasted whole soybeans on unsaturation of milk fatty acids of Holstein and Jersey cows fed control or copper-depleting diets.

end of the preliminary period. The liver biopsy protocol was reviewed and approved by The Ohio State University College of Agriculture Animal Care and Use Committee.

## MATERIALS AND METHODS

### Cows

Twelve Holstein and 12 Jersey cows were allotted to three blocks with four cows of each breed. Block 1 consisted of first lactation cows,  $143 \pm 39$  (mean  $\pm$  SD) DIM at initiation of the preliminary period. Blocks 2 and 3 were older cows at  $94 \pm 20$  DIM at initiation.

### Preexperimental Period

Cows within breed were allotted randomly within blocks to control or copper-depleting diets that were fed for 2 mo to achieve stable or depleted liver copper stores. Diets were 40:15:45 (DM basis) corn silage:alfalfa silage:concentrate (corn, soybean meal base, containing 1.1% supplemental animal-vegetable fat). The control diet was supplemented with 20 mg/kg of copper from  $\text{CuSO}_4$ , whereas the depletion diet contained no supplemental copper, 10 mg/kg of molybdenum from ammonium molybdate, and 0.3% sulfur from  $\text{CaSO}_4$ . Copper status was confirmed by a single liver biopsy (6) at the

### Experimental Period

After the preexperimental period, cows within each copper treatment and breed were assigned randomly to one of two fat treatment sequences. One set of cows (three cows per breed and copper treatment) were fed roasted whole soybeans (15% of DM) for 5 wk and then fed tallow (3% of DM) for 5 wk (Table 1). The other set of cows (three per breed and copper treatment) were fed the reverse sequence. Cows maintained a single copper status throughout the trial. The first 3 wk of each period was used for adaptation and the 4th wk of each period was used for collection of production data and milk and blood composition data. During the 5th wk of each period, the diets were top dressed with 2000 IU/d of supplemental vitamin E (all *rac*- $\alpha$ -tocopheryl acetate) to assess the effect of additional vitamin E on copper, zinc,  $\alpha$ -tocopherol, and retinol concentrations in milk and plasma. Production and milk fat composition data from the 5th wk were not analyzed statistically because of the short duration of the treatment period, the lack of an adaptation period, and because vitamin E treatment was confounded with time.

**Table 1.** Ingredient composition of the concentrate mixes.

	Tallow		Soybean	
	Control copper	Low copper	Control copper	Low copper
	(%)			
Ground shelled corn	33.30	32.50	34.12	33.92
Ground ear corn	20.00	20.00	21.00	21.00
Soybean meal, 44% CP	28.33	28.33	...	...
Roasted whole soybeans <sup>1</sup>	...	...	33.33	33.33
Soybean hulls	0.40	0.40	2.10	2.07
Molasses	2.00	2.00	1.33	1.33
Fat (animal-vegetable blend)	1.10	1.10	0.73	0.73
Fancy bleachable tallow	6.67	6.67	...	...
Limestone	1.86	0.66	1.67	0.44
Dicalcium phosphate	0.68	1.20	0.70	1.20
Calcium sulfate	...	1.50	...	1.50
Sodium bicarbonate	0.50	0.50	0.50	0.50
Urea	1.30	1.30	1.33	1.33
Salt, trace-mineralized <sup>2</sup>	0.90	0.90	0.90	0.90
Magnesium oxide	0.27	0.27	0.27	0.27
Selenium premix (200 mg/kg)	0.33	0.33	0.33	0.33
Zinc oxide	0.10	0.10	0.10	0.10
Copper sulfate	0.02	...	0.02	...
Ammonium molybdate	...	0.004	...	0.004
Vitamins <sup>3</sup>	0.24	0.24	0.24	0.24
Pellet binder	2.00	2.00	1.33	1.33

<sup>1</sup>Whole roasted soybeans added separately to the total ration.

<sup>2</sup>Morton iOFIXT T-M trace-mineralized salt. Guaranteed analysis: NaCl, > 93.0 < 98.0%; all others (mg/kg): Zn, > 3500; Mn, > 2800; Fe, > 1750; Cu, > 350 < 450; I, > 70; Co, > 70.

<sup>3</sup>Vitamin A (4500 IU/g), Vitamin D (1275 IU/g), Vitamin E (18.7 IU/g).

**Table 2.** Chemical composition of the diet DM.

	Tallow		Soybean	
	Control copper	Low copper	Control copper	Low copper
	————— (%) —————			
CP	16.3	16.2	17.4	17.4
NDF	32.5	32.6	33.5	33.2
Fatty acids	4.61	4.76	5.42	5.45
Ca	0.86	0.80	0.87	0.83
P	0.39	0.44	0.41	0.45
	————— (mg/kg) —————			
Cu	36	8.0	33	8.2
Zn	97	102	95	92
Mo	1.9	12.8	0.4	10.1
Fe	254	293	232	258

### Samples and Records

Cows were maintained in tie stalls and were fed the entire ration once daily ad libitum; orts were removed and recorded before feeding. Feed and orts were weighed daily; samples were taken once weekly for DM and were composited for monthly analysis. Adjustments in feed proportions were made weekly according to changes in feed DM. Cows were milked at 0300 and 1300 h; milk yield was measured daily, and samples were taken at a.m. and p.m. milkings once weekly for fat and protein contents by infrared analysis (Dairy Herd Improvement, Inc., Powell, OH). Weekly a.m. and p.m. samples were composited for analysis of copper, zinc,  $\alpha$ -tocopherol, and retinol. Because we previously had observed insignificant differences in fatty acid profiles between a.m. and p.m. milk samples (unpublished), a single a.m. sample was collected once weekly for analysis of milk fatty acid composition. Blood samples were obtained once weekly (2 to 3 h after feeding) in heparinized Vacutainers; plasma was obtained by centrifugation and frozen until analysis.

### Laboratory Analysis

Feed and orts composites were analyzed by a central laboratory (Research and Extension Analytical Laboratory, OARDC, Wooster, OH); for DM (70°C for 24 h), NDF (36); nitrogen in a N analyzer (Foss Heraeus, Hanau, Germany) by combustion of samples in oxygen and measurement of N<sub>2</sub> after reduction of the oxides of N over copper filings (Dumas method) (3); calcium, phosphorous, zinc, molybdenum, and iron were analyzed by inductively coupled plasma spectrograph (ARL, Dearborn, MI) after wet-ashing the samples. Fatty acids in feed and milk fat were extracted, methylated, and determined directly by gas-liquid chromatography (32).

Copper concentration in liver was analyzed as described by Fick et al. (13). Plasma was diluted fivefold with 1% HNO<sub>3</sub> and analyzed directly by atomic absorption (Varian SpectrAA200, Palo Alto, CA) using an auto sampler. Standard curves were developed with appropriate dilutions of reference solutions (1000 mg/L, Fisher Scientific, Pittsburgh, PA). Milk (20 g) was evaporated to dryness in porcelain crucibles and ashed overnight at 600°C. The ash was solubilized with concentrated HNO<sub>3</sub> and samples were diluted to volume (10 ml) with 1% HNO<sub>3</sub>. Samples were analyzed against the standard curves as above. After copper analysis, samples were diluted sevenfold with 1% HNO<sub>3</sub> before analyzing for zinc.

To determine  $\alpha$ -tocopherol and retinol concentrations in plasma, we added 1 ml of reducing solution (10 g of ascorbic acid + 240 ml distilled H<sub>2</sub>O made to 1 L with absolute ethanol) to 0.5 ml of plasma and extracted with 5 ml of hexane; 4 ml of hexane was removed and evaporated under N<sub>2</sub>. Sample volume was taken to 400  $\mu$ l with absolute ethanol and injected (100  $\mu$ l) onto an LC-18 SupelcoSil reversed-phase HPLC column (4.6 mm  $\times$  25 cm, 5  $\mu$  pore size; Supelco #5-8298, Belfonte, PA). The samples were eluted isocratically with metha-

**Table 3.** Fatty acid composition of the diets.

Fatty acid	Tallow		Soybean	
	Control copper	Low copper	Control copper	Low copper
C <sub>14:0</sub>	0.062 <sup>1</sup> (1.34) <sup>2</sup>	0.059 (1.24)	0.014 (0.27)	0.016 (0.30)
C <sub>16:0</sub>	1.00 (21.74)	0.99 (20.85)	0.96 (17.62)	0.95 (17.65)
C <sub>16:1</sub>	0.075 (1.63)	0.082 (1.71)	0.059 (1.09)	0.060 (1.12)
C <sub>18:0</sub>	0.38 (8.33)	0.37 (7.79)	0.21 (3.92)	0.22 (4.01)
<i>trans</i> -C <sub>18:1</sub>	0.058 (1.26)	0.064 (1.34)	0.027 (0.50)	0.029 (0.54)
<i>cis</i> -C <sub>18:1</sub>	0.89 (19.26)	0.97 (20.29)	1.02 (18.76)	1.03 (19.07)
C <sub>18:2</sub>	1.03 (22.37)	1.09 (22.96)	1.88 (34.58)	1.88 (34.88)
C <sub>18:3</sub>	0.27 (5.93)	0.28 (5.95)	0.40 (7.31)	0.40 (7.37)
Other	0.84 (18.14)	0.86 (17.87)	0.85 (15.95)	0.87 (15.06)
Total	4.60 (100)	4.76 (100)	5.42 (100)	5.46 (100)

<sup>1</sup>Percent of the diet DM.

<sup>2</sup>Percent of the total fatty acids.

**Table 4.** Liver copper concentrations ( $\mu\text{g/g}$  of liver DM) after 8 wk of feeding a copper-adequate or a copper-depleting diet.

Treatment	Holstein		Jersey	
		SE		SE
n	10		11	
Control copper	562 <sup>a</sup>	54.9	656 <sup>a</sup>	49.1
Low copper	222 <sup>b</sup>	44.8	272 <sup>b</sup>	44.8

<sup>a,b</sup> $P < 0.0001$ , within a breed.

anol:H<sub>2</sub>O (90:10) at 1.8 ml/min, and UV absorbance was monitored at 300 nm.

Milk fat was extracted and saponified as described by Indyk (17), and  $\alpha$ -tocopherol and retinol were analyzed in the same system as for plasma. The solvent system was methanol:butanol (95:5, vol/vol), developed isocratically.

### Statistics

Liver concentrations of copper were analyzed statistically with a model that included block (2 df), breed (1 df), copper treatment (1 df), breed  $\times$  copper interaction (1 df), and error (18 df). The fat treatment was not yet imposed when biopsies were taken and was not included in the statistical model. The data were analyzed using the general linear model procedure of SAS (30). The experimental design used to analyze production 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 (9). Main plot effects were breed and copper, and fat source was the split plot treatment. The general linear model of SAS (30) 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 (two rows within three replicates = 3 df), C = effect of copper treatment (1 df), B = effect of breed (1 df), B<sup>\*</sup>C = interaction between breed and copper (1 df), R<sup>\*</sup>C<sup>\*</sup>B = error term (6 df) to test B, C and B<sup>\*</sup>C, F = effect of fat treatment (1 df), and C<sup>\*</sup>F (1 df), B<sup>\*</sup>F (1 df), and B<sup>\*</sup>C<sup>\*</sup>F (1 df) = interactions between copper, breed, and fat, and error = error term (27 df) to test F, F<sup>\*</sup>B, F<sup>\*</sup>C, and F<sup>\*</sup>B<sup>\*</sup>C.

The plasma and milk minerals and vitamin data collected during wk 4 of each period (low vitamin E) and during wk 5 of each period (extra vitamin E) were analyzed separately using the same model. The effects of extra vitamin E also were examined by the paired *t*-test (wk 4 vs. wk 5 data within main effect classes). Significance is declared at  $P < 0.05$  unless stated otherwise.

### RESULTS AND DISCUSSION

The chemical composition of the diets is shown in Table 2. Chemical composition was calculated from the individual analyses for corn silage, alfalfa silage, concentrates, and soybeans. The diets containing soybeans had higher proportions of CP, NDF, and fatty acids. The whole roasted soybeans contained more fatty acids (22%) than the reference value (NRC, 24) used to formulate the diets.

As expected, the diets low in copper had lower concentrations of copper than control diets, but the diets were higher (8 and 35 mg/kg) than we calculated (4 and 20 mg/kg) for depleted and control diets, respectively. In general, the copper concentration in the diets was variable because of varying distribution in the concentrate and sampling error. Molybdenum was higher in diets low in copper; the diet low in copper plus soybeans was as calculated, but in the low copper diet plus tallow,

**Table 5.** Treatment means (wk 4 of each treatment period) for DMI, milk yield, and milk composition.

	Jersey				Holstein				SE <sup>1</sup>	SE <sup>2</sup>	Effect <sup>3</sup>
	Tallow		Soybean		Tallow		Soybean				
	Control	Low copper	Control	Low copper	Control	Low copper	Control	Low copper			
DMI, kg/d	13.3	14.1	14.1	14.0	18.1	18.1	18.9	20.1	1.24	1.05	B
DMI, % BW	2.84	3.05	3.02	3.05	3.17	3.04	3.31	3.36	0.25	0.24	
Milk, kg/d	20.0	18.8	20.0	18.0	28.7	27.3	29.2	29.3	2.41	2.03	B
FCM, kg/d	19.3	18.1	19.7	17.0	22.3	20.8	23.0	24.6	1.59	2.09	B
Fat, kg/d	0.76	0.71	0.78	0.66	0.72	0.66	0.75	0.86	0.05	0.09	
Fat, %	3.85	3.75	3.90	3.73	2.53	2.45	2.52	3.01	0.23	0.24	B
Protein, kg/d	0.68	0.66	0.68	0.62	0.92	0.85	0.94	0.92	0.08	0.07	B
Protein, %	3.36	3.49	3.36	3.43	3.22	3.11	3.20	3.13	0.10	0.06	B

<sup>1</sup>SE to test effects of breed, copper, and breed  $\times$  copper.

<sup>2</sup>SE to test effects of fat and fat interactions.

<sup>3</sup>Effects: B = breed ( $P < 0.05$ ).

**Table 6.** Treatment means (wk 4 of each treatment period) for the milk fatty acid profile.

Fatty acid	Jersey				Holstein				SE <sup>1</sup>	SE <sup>2</sup>	Effect <sup>3</sup>
	Tallow		Soybean		Tallow		Soybean				
	Control	Low copper	Control	Low copper	Control	Low copper	Control	Low copper			
	(g/100 g fatty acids)										
C <sub>4:0</sub>	3.38	3.79	3.96	4.04	2.24	2.32	3.49	3.91	0.17	0.10	B, F, B × F
C <sub>6:0</sub>	2.17	2.18	2.83	2.85	1.34	1.23	2.35	2.54	0.086	0.041	B, F, B × F
C <sub>8:0</sub>	1.16	1.15	1.66	1.70	0.71	0.58	1.34	1.33	0.024	0.016	B, F
C <sub>10:0</sub>	2.40	2.34	3.54	3.54	1.56	1.20	2.92	2.63	0.069	0.078	B, F
C <sub>12:0</sub>	2.75	2.61	3.74	3.61	2.08	1.50	3.17	2.83	0.057	0.069	B, F
C <sub>14:0</sub>	10.43	9.97	10.86	9.77	9.27	7.12	10.75	10.13	0.65	0.28	B, C, F, B × F B × C × F
C <sub>14:1</sub>	1.31	1.16	0.82	0.77	1.68	0.82	1.01	0.97	0.094	0.045	F, C × F
C <sub>16:0</sub>	31.35	29.71	27.40	24.52	31.95	30.29	25.58	25.63	4.74	1.14	F
C <sub>16:1</sub>	1.78	1.78	1.04	1.04	3.22	2.80	1.31	1.35	0.057	0.082	B, F, B × F
C <sub>18:0</sub>	11.73	10.96	14.78	15.58	9.66	12.13	12.63	12.27	1.79	2.17	B, F
<i>trans</i> -C <sub>18:1</sub>	4.84	6.64	2.48	3.56	5.39	5.00	4.18	5.27	1.69	1.15	F, B × F
<i>cis</i> -C <sub>18:1</sub>	21.08	21.18	18.81	19.84	24.31	28.03	21.60	20.55	3.31	2.25	B, F
C <sub>18:2</sub>	1.88	2.31	4.33	5.14	2.23	2.50	5.09	5.73	0.53	0.17	F
C <sub>18:3</sub>	0.36	0.44	0.84	0.97	0.42	0.48	0.97	1.03	0.011	0.005	F
CLA <sup>4</sup>	0.70	1.18	0.74	0.87	0.83	0.84	1.02	1.45	0.037	0.069	C, B × F
Other	2.68	2.60	2.17	2.20	3.11	3.16	2.59	2.38	...	...	

<sup>1</sup>SE to test effects of breed, copper, and breed × copper.

<sup>2</sup>SE to test effects of fat and fat interactions.

<sup>3</sup>Effects: B = breed, C = copper, F = fat ( $P < 0.05$ ).

<sup>4</sup>Conjugated linoleic acid.

the molybdenum concentration was higher (13 mg/kg) than we calculated (10 mg/kg).

The fatty acid composition of each diet was calculated from analyses of the individual diet ingredients (Table 3). As expected, the diets that included tallow had higher proportions of saturated fatty acids. In diets with soybeans, the predominant fatty acid was C<sub>18:2</sub>, followed by *cis*-C<sub>18:1</sub> and C<sub>16:0</sub>. Both fat sources provided similar amounts of *cis*-C<sub>18:1</sub>.

The low copper diets fed during the preexperimental period effectively decreased copper storage in liver (Table 4). Cows fed the copper-depleting diet exhibited only 40% of liver copper content of cows fed the control diet ( $P < 0.0001$ ). Jersey cows tended ( $P = 0.13$ ) to have higher copper concentrations in liver than Holstein cows, and this tendency was apparent in both control and copper-depleted cows. Others (12) have reported a difference by breed in copper status. No deleterious effects of copper depletion were apparent at liver biopsy (8 wk of depletion); however, one Holstein cow died after 15 wk of depletion and showed much lower liver copper status at necropsy (67 vs. 134  $\mu\text{g/g}$ ) than at biopsy. Liver copper concentration may have continued to decline in all copper-depleted cows throughout the study. However, copper deficiency in ruminants generally is accepted to occur at liver stores  $<30 \mu\text{g/g}$  of DM (22). One other Holstein cow was lost from the experi-

ment due to causes unrelated to treatment, and liver biopsy was unsuccessful in one Jersey cow.

## Performance

The treatment means for production parameters during wk 4 of fat supplementation periods (without vitamin E) are shown in Table 5. Only breed effects were significant. Intake was higher in Holstein than Jersey cows; however, when expressed as a percentage of BW, there was no difference. Milk and 4% FCM yields were higher for Holstein cows. Milk fat and protein percentages were higher in Jersey cows; however, milk protein yield was higher in Holstein cows.

Treatment effects on milk fatty acid composition are shown in Table 6. Most differences were caused by breed and fat source; depleted copper status decreased C<sub>14:0</sub> and C<sub>16:0</sub>, and increased conjugated linoleic acid (CLA). All fatty acids C<sub>4:0</sub> to C<sub>14:0</sub> and 18:0, were higher in Jersey than in Holstein cows, whereas C<sub>16:1</sub> and *cis*-C<sub>18:1</sub> were lower. Feeding soybeans increased C<sub>4:0</sub> to C<sub>14:0</sub>, C<sub>18:0</sub>, C<sub>18:2</sub>, and C<sub>18:3</sub>, whereas C<sub>14:1</sub>, C<sub>16:0</sub>, C<sub>16:1</sub>, *trans*-C<sub>18:1</sub>, and *cis*-C<sub>18:1</sub> were lower, compared with feeding tallow. Significant breed × fat source interactions occurred for C<sub>4:0</sub>, C<sub>6:0</sub>, C<sub>14:0</sub>, C<sub>16:1</sub>, *trans*-C<sub>18:1</sub>, and CLA. The significant copper status by fat source interaction for C<sub>14:1</sub> and the three-way interaction for C<sub>14:0</sub>

were caused by low amounts of these two fatty acids in milk fat from Holstein cows in low copper status and fed tallow. Because amounts of these two fatty acids are interdependent, the copper  $\times$  fat source effects are unlikely to be random, however, unexplainable.

The treatment means for minerals and fat-soluble vitamins in plasma and milk during wk 4 of each fat treatment period when cows were not supplemented with vitamin E are shown in Table 7. Feeding soybeans decreased plasma copper and zinc, and increased  $\alpha$ -tocopherol in plasma and milk and in milk fat ( $P < 0.07$ ). Zinc concentration was higher in the milk of Jersey cows. Depleted copper status decreased copper in milk, whereas it tended to increase ( $P = 0.11$ ) copper in plasma. A fat source  $\times$  copper status interaction occurred for plasma copper, whereby plasma copper was not influenced by fat source when copper status was adequate (tallow vs. soybean, 1.02 vs. 0.98  $\mu\text{g}/\text{ml}$ ), but plasma copper was higher (1.34 vs. 0.96  $\mu\text{g}/\text{ml}$ ) in depleted copper status when tallow was fed. The three-way interaction also was significant. Plasma retinol also was influenced by a three-way interaction; however, differences were so small as to have questionable physiological significance.

Treatment mean effects on concentrations of copper, zinc,  $\alpha$ -tocopherol, and retinol in plasma and milk during diet supplementation with vitamin E (wk 5, 2000 IU/day) are in Table 8. Feeding soybeans decreased copper and zinc in plasma and copper in milk, whereas  $\alpha$ -tocopherol was increased in milk and milk fat. Copper and zinc in milk were higher and  $\alpha$ -tocopherol tended to be higher ( $P = 0.09$ ) in Jersey cows. Plasma copper concentrations were increased by feeding low copper diets, and this effect was greater in Holstein cows, caus-

ing a breed  $\times$  copper interaction. Conversely, the concentration of copper in milk was higher in Jersey cows, and low copper diets decreased milk copper more in Jersey than Holstein cows, again causing a breed  $\times$  copper interaction. A comparison of vitamin E supplementation on main effects by the paired  $t$ -test showed that supplementing 2000 IU/d of vitamin E increased plasma and milk vitamin E concentrations, and decreased concentrations of zinc in milk. Charmley and Nicholson (7) concluded that supplementing 8000 IU of vitamin E/d for 2 wk increased  $\alpha$ -tocopherol concentration in milk by 20%, although it was not tested statistically. Similarly, Focant et al. (14) reported a 45% increase in concentration of vitamin E in milk when 9616 IU/d was supplemented.

Higher concentrations of short-chain fatty acids and  $\text{C}_{18:0}$  and lower *cis*- $\text{C}_{18:1}$  in milk of Jersey compared with Holstein cows has been observed previously (4, 11, 31). Of considerable interest was the higher concentration of short-chain fatty acids in milk when roasted whole soybeans were fed. The higher proportion of fatty acids synthesized de novo could be partially explained by the lower *trans*- $\text{C}_{18:1}$  in milk from cows fed soybeans; however, differences in the amount of *trans*- $\text{C}_{18:1}$  between fat sources does not seem great enough to explain the effect on de novo synthesis (37). However, fat source could have influenced which *trans* monoene was formed; the *trans*-10 isomer depresses fatty acid synthesis (15). We did not separate the various *trans* isomers in this study. The lower amount of  $\text{C}_{16:0}$  provided by soybeans is reflected in differences of  $\text{C}_{16:0}$  in the milk fat; the effect on differences in milk fat  $\text{C}_{16:0}$  seems even greater than that provided by the diets. Differences in dietary  $\text{C}_{16:0}$  supply possibly affect de novo synthesis.

**Table 7.** Treatment means (wk 4 of each treatment period) for concentrations of trace minerals and fat-soluble vitamins in plasma and milk from cows without vitamin E supplementation.

	Jersey				Holstein				SE <sup>1</sup>	SE <sup>2</sup>	Effect <sup>3</sup>
	Tallow		Soybean		Tallow		Soybean				
	Control	Low copper	Control	Low copper	Control	Low copper	Control	Low copper			
Plasma, $\mu\text{g}/\text{ml}$											
Cu	1.12	1.16	1.06	1.07	0.91	1.53	0.89	0.84	0.11	0.09	C (0.11), F, C $\times$ F, B $\times$ C $\times$ F
Zn	0.89	0.78	0.79	0.81	0.94	0.85	0.84	0.77	0.07	0.04	F (0.07)
Retinol	0.26	0.22	0.24	0.20	0.24	0.13	0.21	0.24	0.03	0.02	B $\times$ C $\times$ F
$\alpha$ -Tocopherol	4.31	4.81	6.84	5.47	6.45	4.50	7.85	5.86	0.84	0.71	C (0.08), F
Milk, $\mu\text{g}/\text{g}$											
Cu	0.20	0.16	0.19	0.17	0.17	0.20	0.18	0.13	0.01	0.02	C, B $\times$ C $\times$ F
Zn	4.57	4.67	4.61	4.70	3.91	3.65	3.90	3.34	0.40	0.31	B
$\alpha$ -Tocopherol	0.25	0.27	0.37	0.44	0.19	0.27	0.32	0.26	0.07	0.07	F
$\alpha$ -Tocopherol, $\mu\text{g}/\text{g}$ fat	6.7	7.7	9.7	13.0	7.7	12.6	13.1	10.3	2.67	2.71	F (0.07)

<sup>1</sup>SE to test effects of breed, copper, and breed  $\times$  copper.

<sup>2</sup>SE to test effects of fat and fat interactions.

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

**Table 8.** Treatment means (wk 5 of each treatment period) for concentrations of trace minerals and fat-soluble vitamins in plasma and milk from cows supplemented with vitamin E (2000 IU/d) for 1 wk.

	Jersey				Holstein				SE <sup>1</sup>	SE <sup>2</sup>	Effect <sup>3</sup>
	Tallow		Soybean		Tallow		Soybean				
	Control	Low copper	Control	Low copper	Control	Low copper	Control	Low copper			
Plasma, $\mu\text{g/ml}$											
Cu	1.05	1.07	0.97	0.98	0.86	1.30	0.81	0.92	0.066	0.08	F, C, B $\times$ C
Zn	0.85	0.82	0.79	0.72	0.91	0.87	0.89	0.76	0.072	0.045	F
Retinol	0.27	0.23	0.23	0.22	0.24	0.17	0.23	0.19	0.029	0.022	
$\alpha$ -Tocopherol	8.26	7.85	9.58	9.16	9.90	7.50	11.2	8.27	1.38	1.05	
Milk, $\mu\text{g/g}$											
Cu	0.21	0.17	0.19	0.14	0.16	0.18	0.14	0.14	0.012	0.013	B, F, B $\times$ C
Zn	4.44	4.50	3.97	4.41	3.22	3.76	3.65	2.92	0.42	0.31	B
$\alpha$ -Tocopherol	0.40	0.34	0.69	0.62	0.32	0.30	0.43	0.48	0.090	0.070	B (0.09), F
$\alpha$ -Tocopherol, $\mu\text{g/g fat}$	12.3	10.8	16.9	16.1	13.9	9.5	16.4	17.8	3.00	2.63	F

<sup>1</sup>SE to test effects of breed, copper, and breed  $\times$  copper.

<sup>2</sup>SE to test effects of fat and fat interactions.

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

Palmitoyl-CoA is the preferred substrate for the initial acylation of  $\alpha$ -glycerophosphate by acyl transferase (18), and the addition of C<sub>16:0</sub> increased de novo fatty acid synthesis in a mammary cell suspension (16). Conversely, a palmitic acid supplement fed to cows decreased C<sub>6:0</sub> to C<sub>14:0</sub> fatty acids of milk fat more than did a stearic acid supplement (23). The higher amounts of unsaturated fatty acids in the mammary gland could have specifically inhibited synthesis of C<sub>16:0</sub> (19).

The lower concentration of *trans*-C<sub>18:1</sub> and *cis*-C<sub>18:1</sub>, and higher C<sub>18:0</sub> in milk fat when soybeans were fed is consistent with more complete ruminal biohydrogenation of soybeans than of tallow. However, feeding soybeans increased C<sub>18:2</sub> and C<sub>18:3</sub> in milk fat by 125%, consistent with earlier reports (34). Possibly, fatty acids of roasted whole soybeans are released more slowly in the rumen than are fatty acids from tallow, thus permitting a more complete biohydrogenation to take place. Simultaneously, slow release would allow some unsaturated fatty acids in soybeans to pass from the rumen without release from the seed. Usually, concentrations of milk CLA are highly correlated with the concentration of *trans*-C<sub>18:1</sub> (28). The higher CLA and lower *trans*-C<sub>18:1</sub> in milk of Holstein cows fed whole soybeans is consistent with apparently higher activity of stearoyl-CoA desaturase in Holstein than Jersey cows (4) because *trans*-11 C<sub>18:1</sub> can be desaturated to produce CLA (10).

Clawson et al. (8) and Pott et al. (27) reported higher concentrations of copper in plasma of animals fed molybdenum; this probably represents accumulation of an insoluble molybdenum-copper complex, which is physiologically unavailable (8). Our data are consistent with the concept that plasma copper is not a reliable pre-

dictor of copper status (12). The decreased concentration of copper in milk in all treatments except Holsteins fed tallow by feeding a copper-depleting diet (Table 7) suggests that concentration of copper in milk is more sensitive than plasma concentrations to depletion. Our data for concentration of copper in milk are in the upper portion of a wide range reported in literature (1). Zinc in milk was higher in Jersey than Holstein cows and, generally, it was within the normal range reported by others (2, 21, 22).

## CONCLUSIONS

Fatty acids released from whole soybeans in the rumen were efficiently biohydrogenated; simultaneously, a portion of the polyunsaturated fatty acids apparently was protected from release and biohydrogenation.

The content of short-chain fatty acids and C<sub>18:0</sub> was higher, and the content of C<sub>16:1</sub> and C<sub>18:1</sub> was lower, in milk from Jersey cows compared with Holstein cows and by feeding roasted whole soybeans compared with feeding tallow. Conversely, feeding roasted whole soybeans increased C<sub>18:2</sub> and C<sub>18:3</sub> in milk fat, compared with feeding tallow. Copper depletion decreased copper in milk; concentration of copper in milk may be a more accurate indicator of the animal's copper status than is plasma copper. In other research in our laboratory, higher copper, C<sub>18:2</sub>, and C<sub>18:3</sub> in milk increased the potential for development of SOF (35); thus feeding roasted whole soybeans and high dietary copper may contribute to development of SOF in milk. Short-term supplementation of 2000 IU of vitamin E daily increased the concentration of this vitamin in milk.

## REFERENCES

- 1 Ammerman, C. B. 1970. Recent developments in cobalt and copper in ruminant nutrition: A review. *J. Dairy Sci.* 53:1097–1107.
- 2 Anderson, R. R. 1992. Comparison of trace elements in milk of four species. *J. Dairy Sci.* 75:3050–3055.
- 3 AOAC. 1990. Protein (Crude) in Animal Feed, Combustion method, 990.03. Official Methods of Analysis. 15th edition. 1st supplement. Association of Official Analytical Chemists International. Arlington, VA.
- 4 Beaulieu, A. D., and D. L. Palmquist. 1995. Differential effects of high fat diets on fatty acid composition in milk of Jersey and Holstein cows. *J. Dairy Sci.* 78:1336–1344.
- 5 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 octadecenoate and linoleate. *Biochem. J.* 130:607–617.
- 6 Chapman, H. L., Jr., D. H. Cox, C. H. Haines, and G. K. Davis. 1963. Evaluation of the liver biopsy technique for mineral nutrition studies with beef cattle. *J. Anim. Sci.* 22:733–737.
- 7 Charmley, E., and J.W.G. Nicholson. 1994. Influence of dietary fat source on oxidative stability and fatty acid composition of milk from cows receiving a low or high level of dietary vitamin E. *Can. J. Anim. Sci.* 74:657–664.
- 8 Clawson, W. J., A. L. Lesperance, V. R. Bohman, and D. C. Layhee. 1972. Interrelationship of dietary molybdenum and copper on growth and tissue composition of cattle. *J. Anim. Sci.* 34:516–520.
- 9 Cochran, W. G., and G. M. Cox. 1950. Pages 234–236 in *Experimental Design*. John Wiley & Sons, Inc., New York.
- 10 Corl, B. A., P. Y. Chouinard, D. E. Bauman, D. A. Dwyer, J. M. Griinari, and K. V. Nurmela. 1998. Conjugated linoleic acid in milk fat of dairy cows originates in part by endogenous synthesis from *trans*-11 octadecenoic acid. *J. Dairy Sci.* 81(Suppl.1):233. (Abstr.)
- 11 DePeters, E. J., J. F. Medrano, and B. A. Reed. 1995. Fatty acid composition of milk fat from three breeds of dairy cattle. *Can. J. Anim. Sci.* 75:267–269.
- 12 Du, Z., R. W. Hemken, and R. J. Harmon. 1996. Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. *J. Dairy Sci.* 79:1873–1880.
- 13 Fick, K. R., L. R. McDowell, P. H. Miles, N. S. Wilkinson, J. D. Funk, and J. H. Conrad. 1979. *Methods of Mineral Analysis for Plant and Animal Tissues*. 2nd ed. Animal Sci. Dept., Univ. of Florida, Gainesville.
- 14 Focant, M., E. Mignolet, M. Marique, F. Clabots, T. Breyne, D. Dalemans, and Y. Larondelle. 1998. The effect of vitamin E supplementation of cow diets containing rapeseed and linseed on the prevention of milk fat oxidation. *J. Dairy Sci.* 81:1095–1101.
- 15 Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K.V.V. Nurmela. 1998. *trans*-Octadecenoic acids and milk fat depression in lactating dairy cows. *J. Dairy Sci.* 81:1251–1261.
- 16 Hansen, H. O., and J. Knudsen. 1987. Effect of exogenous long-chain fatty acids on individual fatty acid synthesis by dispersed ruminant mammary gland cells. *J. Dairy Sci.* 70:1350–1354.
- 17 Indyk, H. E. 1988. Simplified saponification procedure for the routine determination of total vitamin E in dairy products, foods and tissues by high-performance liquid chromatography. *Analyst* 113:1217–1221.
- 18 Kinsella, J. E., and M. Gross. 1973. Palmitic acid and initiation of mammary glyceride synthesis via phosphatidic acid. *Biochim. Biophys. Acta* 316:109–113.
- 19 LaCount, D. W., J. K. Drackley, S. O. Laesch, and J. H. Clark. 1994. Secretion of oleic acid in milk fat in response to abomasal infusions of canola or high oleic sunflower fatty acids. *J. Dairy Sci.* 77:1372–1385.
- 20 Mielke, C. D., and D. J. Schingoethe. 1981. Heat-treated soybeans for lactating cows. *J. Dairy Sci.* 64:1579–1585.
- 21 Miller, W. J. 1970. Zinc nutrition of cattle: a review. *J. Dairy Sci.* 53:1123–1135.
- 22 Mills, C. F. 1987. Biochemical and physiological indicators of mineral status in animals: copper, cobalt and zinc. *J. Anim. Sci.* 65:1702–1711.
- 23 Noble, R. C., W. Steele, and J. H. Moore. 1969. The effects of dietary palmitic and stearic acids on milk fat composition in the cow. *J. Dairy Res.* 36:375–381.
- 24 NRC. 1989. *Nutrient Requirements of Dairy Cattle*. 6th Rev. Ed. National Research Council. Nat. Acad. Sci., Washington, DC.
- 25 Palmquist, D. L. 1997. Spontaneous oxidized flavor in winter milk is associated with increased linoleic acid content from feeding whole soybeans. *Proc., Milk fat: Nutrition and Product Development. Scandinavian Lipidforum and the Swedish Nutrition Foundation. Malmö. (Abstr.)*
- 26 Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* 76:1753–1771.
- 27 Pott, E. B., P. R. Henry, P. V. Rao, E. J. Hinderberger, Jr., and C. B. Ammerman. 1999. Estimated relative bioavailability of supplemental inorganic molybdenum sources and their effect on tissue molybdenum and copper concentrations in lambs. *Anim. Feed Sci. Technol.* 79:107–117.
- 28 Precht, D., and J. Molkenin. 1997. Effect of feeding on conjugated *cis*  $\Delta$ 9, *trans*  $\Delta$ 11-octadecadienoic acid and other isomers of linoleic acid in bovine milk fats. *Nahrung* 41:330–335.
- 29 Richardson, T., and M. Korycka-Dahl. 1983. Lipid oxidation. Pages 241–363 in *Developments in Dairy Chemistry—2. Lipids*. P. F. Fox, ed. Applied Science, New York, NY.
- 30 SAS Software: Changes and Enhancements through Release 6.12. 1997. SAS Inst., Inc., Cary, NC.
- 31 Stull, J. W., and W. H. Brown. 1964. Technical note: Fatty acid composition of milk. II. Some differences in common dairy breeds. *J. Dairy Sci.* 47:1412.
- 32 Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J. Agric. Food Chem.* 36:1202–1206.
- 33 Thompson, E. H., C. E. Allen, and R. J. Meade. 1973. Influence of copper on stearic acid desaturation and fatty acid composition in the pig. *J. Anim. Sci.* 36:868–873.
- 34 Tice, E. M., M. L. Eastridge, and J. L. Firkins. 1994. Raw soybeans and roasted soybeans of different particle sizes. 2. Fatty acid utilization by lactating cows. *J. Dairy Sci.* 77:166–180.
- 35 Timmons, J. S. 1999. Contribution of dietary roasted soybeans and milk components to the development of spontaneous oxidized milk flavor. M.S. Thesis. The Ohio State Univ., Columbus.
- 36 VanSoest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- 37 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.