

Effects of soybean oil and dietary copper on ruminal and tissue lipid metabolism in finishing steers^{1,2}

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ABSTRACT: An experiment was conducted to determine the effects of Cu and soybean oil (SBO) supplementation on ruminal and tissue lipid metabolism and carcass characteristics in finishing steers. Sixty Angus steers (369.0 ± 10.1 kg) were stratified by weight and randomly assigned to treatments in a 2×2 factorial arrangement, with factors being 0 or 20 mg of supplemental Cu/kg DM from Cu sulfate and 0 or 4% SBO. Steers were fed a high-concentrate basal diet that contained 5.3 mg Cu/kg DM. Average daily gain and feed intake were reduced ($P < 0.01$) by SBO but were not affected by Cu. Gain:feed ratio was not affected by treatment. Liver Cu concentrations were higher ($P < 0.01$) in steers receiving supplemental Cu and lower ($P < 0.04$) in SBO-supplemented steers. Copper supplementation tended to reduce ($P < 0.12$) and SBO supplementation

tended to increase ($P < 0.11$) serum cholesterol concentrations. Backfat depth was reduced ($P < 0.10$) by Cu and SBO supplementation. Marbling scores and longissimus muscle lipid content were not affected by Cu supplementation; however, SBO supplementation reduced ($P < 0.01$) marbling scores. Longissimus muscle polyunsaturated fatty acids tended to be increased ($P < 0.14$) in Cu-supplemented steers. Longissimus muscle C18-conjugated dienes and the 18:1 *trans* isomer were increased ($P < 0.05$) in SBO-supplemented steers. Ruminal fluid 18:3 was increased ($P < 0.05$) and the 18:1 *trans* isomer was decreased ($P < 0.05$) in Cu-supplemented steers. These results indicate that as little as 20 mg of supplemental Cu/kg DM can reduce backfat and may alter lipid metabolism in steers fed high-concentrate diets.

Key Words: Cholesterol, Copper, Fatty Acids, Steers

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Introduction

It was first demonstrated by Reiser (1951) that unsaturated fatty acids in the rumen are hydrogenated to saturated fatty acids by ruminal microorganisms. This biohydrogenation allows for only small amounts of dietary unsaturated fatty acids to bypass the rumen and be absorbed by the animal. Therefore, the lipid content of ruminant tissue contains a high proportion of saturated fatty acids (Garret et al., 1976) and intermediates from the biohydrogenation process (e.g., the 18:1 *trans*

isomer; Christie, 1981) compared with nonruminant species.

Recently, Engle et al. (2000) reported that adding 20 or 40 mg Cu/kg DM to growing and finishing diets increased the proportion of polyunsaturated fatty acids (18:2 and 18:3) and decreased the 18:1 *trans* isomer in longissimus muscle. This suggests that Cu supplementation may have altered microbial biohydrogenation of fatty acids in the rumen, thus allowing for a greater amount of unsaturated fatty acids to bypass the rumen and be absorbed from the lower gut. The present study was conducted to determine whether dietary Cu would inhibit the biohydrogenation of supplemental fat (soybean oil, high in polyunsaturated fatty acids) allowing for a higher concentration of polyunsaturated fatty acids to accumulate in longissimus muscle, and adipose tissue.

Materials and Methods

Sixty Angus steers (369.0 ± 10.1 kg) were used in this experiment. Care and handling of the animals and sampling procedures were approved by the North Carolina State University Animal Care and Use Committee.

¹Use of trade names in this publication does not imply endorsement by the North Carolina Agric. Res. Serv. or criticism of similar products not mentioned.

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Table 1. Composition of finishing diet (% DM)^a

Item	No added Cu		Added Cu	
	No SBO	SBO	No SBO	SBO
Corn	86.8	82.8	86.8	82.8
Soybean meal (48% CP)	6.0	6.0	6.0	6.0
Cottonseed hulls	5.0	5.0	5.00	5.0
Soybean oil	—	4.0	—	4.0
Calcium sulfate	0.80	0.80	0.80	0.80
Urea	0.75	0.75	0.75	0.75
Calcium carbonate	0.40	0.40	0.40	0.40
Salt	0.20	0.20	0.20	0.20
Vitamin premix ^b	0.02	0.02	0.02	0.02
Cu, 20 mg/kg DM	—	—	+ ^e	+
Mineral premix ^c	+	+	+	+
Monensin ^d	+	+	+	+

^aEther extract of diets without supplemental SBO averaged 3.5% of DM.

^bContained per kilogram of premix: 6,600,000 IU of vitamin A; 3,520,000 IU of vitamin D; and 6,600 IU of vitamin E.

^cProvided per kilogram of diet: 30 mg of Zn as ZnSO₄, 20 mg of Mn as MnSO₄, 0.5 mg of I as Ca(IO₃)₂(H₂O), 0.1 mg of Co as CoCO₃, and 0.1 mg of Se as Na₂SeO₃.

^dProvided 30 mg of monensin/kg DM.

^eLess than 0.01.

Steers were obtained from the North Carolina State University cow-calf facility. Following weaning, steers were vaccinated with Cattle Master 4 (Pfizer Animal Health, Exton, PA) and Vision 7 (Bayer, Shawnee Mission, KS), wormed with Safe Guard (Hoechst-Roussel, Sommerville, NJ), and fed a corn silage-based growing diet adequate in Cu for approximately 100 d.

Upon initiation of the present study, steers were weighed on two consecutive days, implanted with Synovex-Plus (Fort Dodge Animal Health, Fort Dodge, IA), bled via jugular venipuncture, and liver biopsied. Steers were allotted to one of four groups based on body weight. Groups were randomly assigned to treatments in a 2 × 2 factorial arrangement, with factors being 0 or 20 mg of supplemental Cu/kg DM from Cu sulfate (CuSO₄) and 0 or 4% soybean oil (SBO). Steers were gradually switched (over an 8-d period) to a high-concentrate finishing diet (Table 1; diets not supplemented with Cu contained 5.2 mg Cu/kg DM, 52.1 mg Zn/kg DM, 50.9 mg Fe/kg DM, 0.29% S, and 0.59 mg Mo/kg DM). Diets were formulated to meet or exceed all nutrient requirements for finishing steers with the exception of Cu (NRC, 1996). Diets were fed once daily in the morning in amounts adequate to allow ad libitum access to feed. Steers were housed in 12-animal, covered, slotted-floor pens (10 m × 5 m) equipped with individual Calan gate feeders (American Calan, Northwood, NH).

Jugular blood samples were collected in heparinized and unheparinized Vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) on d 0, 28, 56, and 84 and before shipment for slaughter and were analyzed for plasma Cu and serum total cholesterol concentrations. A liver biopsy was obtained, as described previously (Engle and Spears, 2000a), on d 0 and 84, and a liver sample was obtained postmortem. Blood and liver samples were immediately stored in ice and transported back to the laboratory. Blood samples were centrifuged

at 1800 × g at 5°C for 30 min, and plasma and serum were removed. Plasma, serum, and liver samples were frozen at -20°C until analyzed.

On d 90, eight steers per treatment with similar feed intakes and body weights were fed at staggered intervals in order to obtain ruminal fluid samples at 2 h after feeding. Ruminal fluid was collected from steers using a stomach tube. Ruminal contents were allowed to flow out of the tube for approximately 5 s, and then a 1-L sample of ruminal contents was collected in an Erlenmeyer flask and strained through eight layers of cheesecloth. Strained ruminal fluid was placed on ice and transported back to the laboratory and frozen at -80°C until analyzed for fatty acid composition.

The heaviest six steers per treatment were slaughtered on d 96, and remaining nine steers per treatment were slaughtered on d 130 of the study. Final weights were obtained on two consecutive days, and steers were then transported approximately 320 km to a commercial abattoir and slaughtered after an overnight fast. Hot carcass weight was determined on the day of slaughter. Carcass grading was conducted by a certified USDA grader 48 h after slaughter. After carcass grading, a longissimus muscle sample, approximately 1.5 cm in depth and encompassing the entire ribeye surface area, was sliced from the right side of the carcass dorsally from the area of backfat measurement to the spine of the carcass (approximate weight, 100 g). Samples were placed in plastic bags and immediately chilled on ice. On arrival at the laboratory, samples were frozen at -80°C until analyzed for total lipid, fatty acid, cholesterol, and Cu concentrations.

Analytical Procedures. Plasma samples were diluted 1:3 (vol:vol) in deionized water, and Cu concentrations were determined by flame atomic absorption spectrophotometry (Model AA-6701F, Shimadzu, Japan). Serum samples were analyzed for total cholesterol concen-

trations using the method described by Sigma Chemical Co. (1995).

Longissimus muscle (with visible external fat dissected), backfat, and liver tissue samples were thawed at room temperature. Longissimus muscle, backfat, and the final liver samples obtained at slaughter were diced into small pieces, mixed thoroughly, and then randomly subsampled. Aliquots of longissimus muscle, final liver subsamples, and liver biopsy samples in their entirety were dried at 100°C for 48 h, weighed, and then analyzed for Cu (Engle et al., 2000). Triplicate 1-g subsamples of longissimus muscle and backfat, and duplicate 1-g subsamples of final liver samples, were used for lipid extraction (Engle et al., 2000). The percentage of lipid was determined for longissimus muscle and liver tissue. Total longissimus muscle cholesterol concentration was determined by the enzymatic method of Allain et al. (1974) as modified by Salé et al. (1984). Lipid in ruminal fluid was extracted in duplicate, as described by Fellner et al. (1995).

Methyl ester derivatives of the fatty acids extracted from longissimus muscle, backfat, and ruminal fluid samples were prepared in duplicate using a combination of NaOCH₃ followed by HCl/CH₃OH as described by Kramer et al. (1997). Methylated ruminal lipids were further purified by TLC using the ruminal lipid extract reconstituted in 100 µL of hexane. The extract was spotted on silica gel G plates (20 × 20 cm, 250 µm; Fisher Scientific, Raleigh, NC) with a micropipette. The TLC plates were developed in 1,2 dichloroethane solvent for 30 min in a tank containing butylated hydroxytoluene crystals, to prevent oxidation. Plates were then dried under N₂ gas for 10 min. Fatty acid methyl ester bands were identified and scraped into funnels plugged with glass wool. The samples were washed with 10 mL of CHCl₃ to remove the fatty acid methyl esters from the silica gel and brought to dryness under N₂ gas. Fatty acid methyl esters were reconstituted with 200 µL of hexane in vials ready for chromatographic analysis. Fatty acid composition of longissimus muscle, backfat, and ruminal fluid samples were determined via GLC (Engle et al., 2000).

Statistical Analysis. Statistical analysis of data was performed by analysis of variance for a completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Performance, liver Cu concentrations, blood variables, carcass data, and longissimus muscle and adipose tissue fatty acid composition data were analyzed using repeated measures with the model including variation due to treatment (Cu and SBO), the two-way interaction, time, and treatment × time interactions. There were no time effects or treatment × time interactions for carcass data, and longissimus muscle and adipose tissue fatty acid composition. The model for ruminal fluid included variation due to Cu, SBO, and the two-way interaction. Significance was declared at $P < 0.10$.

Results and Discussion

Performance was not affected by Cu supplementation over the entire finishing period. These results are in agreement with an earlier study in which Cu supplementation at 10 or 20 mg Cu/kg DM to steers fed a high-concentrate finishing diet had no effect on performance relative to unsupplemented controls (Engle and Spears, 2000b). In contrast to the present study, Cu supplementation to finishing steers at 20 or 40 mg Cu/kg DM reduced ($P < 0.05$) gain, feed intake, and gain:feed relative to unsupplemented controls (Engle and Spears, 2000a). In other studies from our laboratory, Cu supplementation to finishing cattle has increased performance (Ward and Spears, 1997; Engle and Spears, 1999). Conflicting performance results exist for cattle consuming high-concentrate diets supplemented with Cu. The reason for the discrepancy between results is not clear. There are many factors that could potentially affect an animal's response to Cu supplementation, such as initial Cu status of the animal, duration and concentration of Cu supplementation, the absence or presence of dietary Cu antagonists (S and Mo), environmental and health factors, and breed differences in Cu metabolism.

Final ADG and DMI were decreased by SBO supplementation. The reductions in ADG and DMI were observed by d 28 and at subsequent 28-d intervals (Table 2). The decrease in performance in SBO-supplemented steers may have been due to the high unsaturated fatty acid content of SBO (84.1% unsaturated fatty acids; data not shown) affecting rumen fermentation. Fat sources high in unsaturated fatty have been shown to decrease feed intake (Czerkawski and Clapperton, 1984) and inhibit fiber digestion (Palmquist and Jenkins, 1980; Ikwuegbu and Sutton, 1982; Chalupa et al., 1984, 1986) to a greater extent than fat sources high in saturated fatty acids. In contrast, Brandt and Anderson (1990) observed an ADG ($P < 0.05$) and a gain:feed ratio ($P < 0.01$) in steers consuming a high-concentrate diet supplemented with 3.5% SBO that were higher than with the unsupplemented controls. The discrepancy between the two studies may be due partly to the difference in roughage and oil concentration of the basal diets. In the present study, the roughage source was cottonseed hulls included at 5% of the diet DM; the ether extract of the basal diet was 3.5%. Brandt and Anderson (1990) fed a high concentrate, flaked milo diet that contained 4% alfalfa hay and 4% corn silage as roughage sources, with the basal diet containing 1.64% ether extract. It has been suggested that increasing the roughage content of the diet may decrease the inhibitory effects of unsaturated fatty acids on ruminal fermentation (Mir, 1988; Doreau et al., 1991). Furthermore, ruminal microorganisms seem to be able to tolerate only a small amount (3 to 5%) of added fat (Hatch et al., 1972; Palmquist and Jenkins, 1980). The total fat content of the SBO-supplemented diet in the present study was 7.5%, whereas the total fat content of the

Table 2. Effects of copper and soybean oil (SBO) supplementation on performance of finishing steers

Item	Dietary treatment				SEM	Significance ($P <$)		
	No added Cu		Added Cu ^a			Cu	SBO	Cu \times SBO
	No SBO	SBO ^b	No SBO	SBO ^b				
Body wt, kg								
d 0	369	369	369	369	10	NS ^c	NS	NS
d 28	421	411	425	412	10	NS	0.09	NS
d 56	474	453	483	458	11	NS	0.03	NS
d 84	510	484	517	485	12	NS	0.02	NS
Final	568	538	558	540	13	NS	0.08	NS
ADG, kg								
d 0–28	1.84	1.51	1.98	1.53	0.12	NS	0.01	NS
d 29–56	1.92	1.48	2.10	1.68	0.14	NS	0.01	NS
d 57–84	1.26	1.10	1.21	0.94	0.09	NS	0.07	NS
Final	1.60	1.41	1.62	1.41	0.08	NS	0.01	NS
DMI, kg								
d 0–28	10.01	9.42	10.41	9.12	0.32	NS	0.01	NS
d 29–56	9.75	7.80	10.29	8.31	0.39	NS	0.001	NS
d 57–84	8.70	8.63	8.82	7.52	0.32	NS	0.02	NS
Final	9.61	8.61	9.77	8.53	0.26	NS	0.001	NS
Gain:feed								
d 0–28	0.18	0.16	0.19	0.17	0.01	NS	NS	NS
d 29–56	0.20	0.19	0.20	0.20	0.02	NS	NS	NS
d 57–84	0.15	0.13	0.14	0.13	0.01	NS	NS	NS
Final	0.17	0.16	0.17	0.17	0.01	NS	NS	NS

^a20 mg of Cu/kg DM as CuSO₄.

^bSoybean oil added at 4% of the diet DM.

^cNS = Not significant ($P > 0.10$).

SBO-supplemented treatment fed by Brandt and Anderson (1990) contained 5.1% fat.

There was a treatment \times time interaction for plasma ($P < 0.10$) and liver ($P < 0.04$) Cu concentrations. Final plasma Cu concentrations were higher ($P < 0.09$) in steers receiving supplemental Cu (Table 3). Liver Cu concentrations were higher in Cu-supplemented steers by d 84 and after slaughter (Table 3). Soybean oil sup-

plementation decreased final liver Cu concentrations (Table 3). Steers fed the supplemental SBO had lower feed intakes, which could explain part of the reduction in liver Cu concentrations (Table 2). There are also data indicating that increasing dietary fat concentrations decreases divalent cation absorption in rats (Ebesh et al., 1999) and cattle (Rahnema et al., 1994). Plasma and liver Cu concentrations of all treatments remained

Table 3. Effects of copper and soybean oil (SBO) supplementation on plasma and liver copper concentrations in steers

Item	Dietary treatment				SEM	Significance ($P <$)		
	No added Cu		Added Cu ^a			Cu	SBO	Cu \times SBO
	No SBO	SBO ^b	No SBO	SBO ^b				
Plasma Cu, mg/L								
d 0	0.91	0.89	0.93	0.93	0.04	NS ^c	NS	NS
d 28	0.90	0.90	0.90	0.91	0.05	NS	NS	NS
d 56	0.89	0.92	0.95	0.94	0.04	NS	NS	NS
d 84	0.88	0.95	0.95	0.98	0.03	NS	NS	NS
Final	0.91	1.0	1.2	1.3	0.06	0.09	NS	NS
Liver Cu, mg/kg DM								
d 0	79.8	69.4	72.6	86.6	10.1	NS	NS	NS
d 84	55.8	57.3	137.3	133.2	12.2	0.01	NS	NS
Final	58.8	43.4	221.8	200.8	11.2	0.001	0.04	NS

^a20 mg of Cu/kg DM as CuSO₄.

^bSoybean oil added at 4% of the diet DM.

^cNS = Not significant ($P > 0.10$).

Table 4. Effects of copper and soybean oil (SBO) supplementation on total serum cholesterol concentrations (mg/dL) in steers

Item	Dietary treatment				SEM	Significance ($P <$)		
	No added Cu		Added Cu ^a			Cu	SBO	Cu × SBO
	No SBO	SBO ^b	No SBO	SBO ^b				
d 0	73.4	73.6	77.0	71.4	3.4	NS ^c	NS	NS
d 28	73.2	72.0	75.1	71.2	2.8	NS	NS	NS
d 56	79.2	82.2	78.9	80.1	2.9	NS	NS	NS
d 84	86.2	90.2	85.2	87.5	3.9	NS	NS	NS
Final	95.3	98.5	85.2	93.5	3.7	0.12	0.11	NS

^a20 mg of Cu/kg DM as CuSO₄.

^bSoybean oil added at 4% of the diet DM.

^cNS = Not significant ($P > 0.10$).

above concentrations considered to be indicative of Cu deficiency (liver < 20 mg of Cu/kg DM and plasma $< .6$ mg of Cu/L; Mills, 1987).

There was a trend ($P < 0.15$) for a treatment × time effect for serum cholesterol concentrations. Serum cholesterol concentrations were unaffected through d 84 but tended to be lower ($P < 0.12$) in Cu-supplemented steers at the end of the study (Table 4). Engle et al. (2000) reported that Cu supplementation (20 or 40 mg Cu/kg DM) reduced serum cholesterol concentrations in finishing steers. Furthermore, the addition of much higher concentrations of Cu (125 to 250 mg Cu/kg DM) to broiler diets reduced plasma cholesterol concentrations (Pesti and Bakalli, 1996). Steers receiving SBO tended to have higher ($P < 0.11$) serum cholesterol concentrations at the end of the study (Table 4). This is consistent with findings by Jenkins (1990) that fat supplementation to steers increased plasma cholesterol concentrations relative to unsupplemented controls.

Cholesterol concentrations of longissimus muscle were not affected by Cu or SBO supplementation (Table 5). This is in contrast to previous research in which Cu supplementation at 20 or 40 mg Cu/kg DM decreased cholesterol concentrations of longissimus muscle (Engle

et al., 2000). Cholesterol concentrations of longissimus muscle in control steers from the present study were 22% lower than cholesterol concentrations of longissimus muscle in control steers in the previous study. This may partly explain the lack of response to Cu supplementation in the present study. Furthermore, steers were supplemented with Cu for a longer duration by Engle et al. (2000) than in the present study. Longissimus muscle Cu and total lipid concentrations were not affected by treatment. Total liver lipid was not affected by dietary addition of Cu or SBO.

Backfat depth was decreased ($P < 0.10$) by Cu supplementation (Table 6), but marbling scores were similar between Cu-supplemented and unsupplemented steers. Consistent with the present study, Engle and Spears (2000b) also reported that Cu supplementation reduced backfat depth without affecting ADG or marbling in finishing steers. In some studies, (Ward and Spears, 1997; Engle and Spears, 1999) Cu supplementation to finishing diets has increased ADG but reduced backfat depth. Marbling score, dressing percentage, hot carcass weight, backfat depth, kidney, pelvic, and heart fat, yield grade, and quality grade were decreased by SBO supplementation (Table 6), which is consistent with the

Table 5. Effects of copper and soybean oil (SBO) supplementation on tissue cholesterol, lipid, and copper concentration in steers

Item	Dietary treatment				SEM	Significance ($P <$)		
	No added Cu		Added Cu ^a			Cu	SBO	Cu × SBO
	No SBO	SBO ^b	No SBO	SBO ^b				
Longissimus muscle								
DM, %	29.8	28.8	30.1	29.8	1.1	NS ^c	NS	NS
Cholesterol, mg/100 g wet wt	58.3	58.4	56.2	55.9	2.9	NS	NS	NS
Lipid, % wet wt	4.3	4.1	4.0	4.2	0.3	NS	NS	NS
Copper, mg/kg DM	3.6	3.4	3.5	3.3	0.6	NS	NS	NS
Liver								
% DM	29.1	28.7	27.2	29.4	0.5	NS	NS	NS
Lipid, % wet wt	3.2	2.9	2.8	2.9	0.4	NS	NS	NS

^a20 mg of Cu/kg DM as CuSO₄.

^bSoybean oil added at 4% of the diet DM.

^cNS = Not significant ($P > 0.10$).

Table 6. Effects of copper and soybean oil (SBO) supplementation on carcass characteristics in steers

Item	Dietary treatment				SEM	Significance ($P <$)		
	No added Cu		Added Cu ^a			Cu	SBO	Cu \times SBO
	No SBO	SBO ^b	No SBO	SBO ^b				
Marbling ^c	6.0	5.4	5.8	4.8	0.24	NS ^g	0.01	NS
Dressing percentage	58.9	57.9	60.0	57.9	0.60	NS	0.09	NS
Hot carcass wt, kg	334	311	341	313	6	NS	0.01	NS
12th-rib backfat, cm	1.24	0.98	0.96	0.90	0.09	0.10	0.10	NS
KPH, % ^d	2.4	2.2	2.6	2.3	0.12	NS	0.05	NS
USDA yield grade	2.9	2.4	3.0	2.6	0.15	NS	0.04	NS
LMA, cm ² ^e	80.8	84.3	85.8	81.1	1.8	NS	NS	0.03
USDA quality grade ^f	17.8	16.8	17.5	16.3	0.26	NS	0.01	NS

^a20 mg of Cu/kg DM as CuSO₄.

^bSoybean oil added at 4% of the diet DM.

^c4 = slight; 5 = small; 6 = modest.

^dKidney, pelvic, and heart fat.

^eLongissimus muscle area at the 12th–13th rib interface.

^fSelect⁺ = 16; Choice⁻ = 17; Choice = 18.

^gNS = Not significant ($P > 0.10$).

decrease in performance observed in SBO-supplemented steers (Table 2). There was a Cu \times SBO interaction for longissimus muscle area (LMA). The addition of Cu without SBO increased LMA, whereas addition of both Cu and SBO decreased LMA.

Recent studies (Engle and Spears, 1999; Engle et al., 2000) suggested that dietary Cu may affect microbial biohydrogenation of fatty acids in the rumen. Adding 20 or 40 mg Cu/kg DM to growing and finishing cattle diets increased polyunsaturated fatty acid proportions of total fat in longissimus muscle (Engle et al., 2000a). The 18:1 *trans* isomer, an intermediate of biohydrogenation (Kepler et al., 1966; Christie, 1981), was reduced in longissimus muscle of Cu-supplemented steers, suggesting an effect of Cu on biohydrogenation. Biohydrogenation of 18:2 initiates with the isomerization of the *cis*-9, *cis*-12 molecule into *cis*-9 *trans*-11 18:2 (a conjugated linoleic acid) as the first intermediate (Kepler et al., 1966). The subsequent step is a reduction of the *cis*-9 double bond, resulting in *trans* 18:1 (n-7) as the second intermediate. This step involves a reductase using electron donors. The final step is another reduction, resulting in 18:0. Copper may inhibit biohydrogenation of 18:2 by interfering with the formation of an electronegative center involved in the hydrogen transfer in the isomerization of 18:2 (Kepler et al., 1971). Another suggestion has been that iron is involved in the activity of the reductase, but Cu or other metal ions are not (Hughes et al., 1982). However, Cu may serve as an electron carrier in the reduction process (Hunter et al., 1976). It was therefore hypothesized that, if Cu supplementation decreased ruminal biohydrogenation of unsaturated fatty acids, then supplementing SBO (high in unsaturated fatty acids) to the diet of Cu-supplemented steers would further increase unsaturated fatty acids and decrease saturated fatty acids and the 18:1 *trans* isomer content of ruminal fluid, longissimus muscle, and backfat.

In the present study, Cu supplementation decreased ($P < 0.05$) the 18:1 *trans* isomer and increased ($P < 0.05$) 18:3 percentages in ruminal fluid (Table 7). The majority of lipids in the rumen are bound to feed particles; therefore, the fatty acid profile of rumen fluid is primarily that of liquid-associated microorganisms (Lough, 1970). Total 18:1 was decreased ($P < 0.10$) in Cu-supplemented steers, primarily due to the decrease in the 18:1 *trans* isomer. Soybean oil supplementation decreased 15:0, 17:0, and the 18:1 *cis* isomer (Table 7). As expected, SBO (51.9% 18:2; data not shown) supplementation increased ($P < 0.01$) the 18:2 percentage in ruminal fluid. However, the 18:0 percentage remained unchanged. This was unexpected because an increase in SBO supplementation should result in an increase in the substrate for microbial biohydrogenation (Christie, 1981). Studies have shown that feeding a high-concentrate diet decreased biohydrogenation of dietary unsaturated fatty acids (Latham et al., 1972; Kemp et al., 1981; Palmquist and Schanbacher, 1991), due to a decrease in the lipolytic bacterial population. With a reduction in lipolytic bacteria, a free carboxyl group on fatty acids (which is a prerequisite for the initial isomerization step in biohydrogenation) is not generated (Jenkins, 1994), thus allowing unsaturated fatty acids to pass through the rumen unaltered. Ruminal fluid triglyceride concentrations were not measured in the present study. Furthermore, the fatty acid profile of lipid absorbed onto ruminal particulate matter was not determined.

There was a Cu \times SBO interaction for concentrations of 18:0 ($P < 0.03$), the 18:1 *cis* isomer ($P < 0.05$), C18-conjugated dienes ($P < 0.03$), total unsaturated ($P < 0.10$), and total saturated ($P < .10$) fatty acids in rumen fluid (Table 7). Copper supplementation increased the percentages of the 18:1 *cis* isomer, C18-conjugated dienes, and total unsaturated fatty acids in steers unsupplemented with SBO but increased 18:0 and total

saturated fatty acid percentages in SBO-supplemented steers. The Cu \times SBO interaction suggests that Cu supplementation may have decreased biohydrogenation in non-SBO-fed steers but increased ruminal biohydrogenation in SBO-fed steers. However, the absence of a Cu \times SBO interaction for 18:2 makes these data difficult to interpret.

Longissimus muscle polyunsaturated fatty acids ($P < 0.14$) and the 16:1 *cis* isomer tended ($P < 0.12$) to be increased by Cu supplementation (Table 8). The 18:1 *trans* isomer tended ($P < 0.12$) to be decreased by Cu supplementation (Table 8), indicating that Cu may have also decreased ruminal biohydrogenation of unsaturated fatty acids. Copper increased ($P < 0.03$) 22:0 and decreased ($P < 0.10$) 18:0 in longissimus muscle. Soybean oil supplementation decreased the 16:1 *cis* ($P < 0.01$) and 18:1 *cis* isomers ($P < .08$) and increased the 18:1 *trans* isomer ($P < 0.02$) and C18-conjugated dienes ($P < 0.05$), and it tended to increase 18:0 ($P < 0.11$). There were no Cu \times SBO interactions.

Subcutaneous adipose tissue percentages of 16:0 were increased ($P < 0.06$) and the 18:1 *cis* isomer was decreased ($P < 0.08$) in steers receiving supplemental SBO (data not shown). There were no Cu effects or Cu \times SBO interactions for s.c. adipose tissue fatty acids.

The present study agrees with earlier research (Ward and Spears, 1997; Engle et al., 2000; Engle and Spears, 2000b) indicating that dietary Cu decreases backfat in finishing cattle. Performance and cholesterol concentrations of longissimus muscle were not altered by Cu supplementation. Soybean oil supplementation at 4%

DM to a basal diet containing 3.5% fat decreased DMI and ADG. The reduction in performance was probably due to the high level of total dietary fat (7.5% ether extract) as well as to the high degree of unsaturated fatty acids in SBO. It seems from these results that Cu supplementation may alter lipid biohydrogenation in the absence of high dietary lipid, as indicated by an increase in 18:3 and a decrease in 18:1 *trans* isomer in ruminal fluid, as well as a decrease in 18:0, a trend for a decrease in the 18:1 *trans* isomer, and a trend for an increase in polyunsaturated fatty acid percentages in longissimus muscle. However, in the presence of high dietary lipid (SBO), Cu supplementation seems to enhance lipid biohydrogenation in the rumen. Further research is needed to determine the mechanism by which Cu alters lipid metabolism in cattle.

Implications

Supplementation of 20 mg of copper per kilogram of dry matter can decrease carcass backfat without altering marbling scores or body weight gain. A decrease in carcass backfat would reduce the amount of trimming required at slaughter. Copper supplementation to diets not containing supplemental soybean oil seems to decrease ruminal biohydrogenation of polyunsaturated fatty acids, but, in the presence of soybean oil, copper appears to increase biohydrogenation. Further research is needed to determine conclusively if Cu alters the biohydrogenation of polyunsaturated fatty acids.

Table 7. Effects of copper and soybean oil (SBO) supplementation on fatty acid composition of rumen fluid of finished steers^a

Fatty acid ^d	Dietary treatment				SEM	Significance ($P <$)		
	No added Cu		Added Cu ^b			Cu	SBO	Cu \times SBO
	No SBO	SBO ^c	No SBO	SBO ^c				
	g/100 g							
14:0	1.41	1.65	1.41	1.96	.47	NS ^e	NS	NS
15:0	1.09	0.93	1.16	0.88	0.11	NS	0.07	NS
16:0	21.80	22.58	22.81	21.48	1.00	NS	NS	NS
17:0	0.71	0.58	0.71	0.58	0.10	NS	0.09	NS
18:0	50.11	48.26	50.35	52.49	2.01	NS	NS	0.03
Total 18:1	18.50	19.17	17.47	15.50	1.10	.10	NS	NS
18:1 <i>trans</i>	13.62	15.17	12.71	11.99	1.00	.05	NS	NS
18:1 <i>cis</i>	4.88	4.01	4.75	3.51	0.90	NS	0.01	0.05
18-conjugated dienes	0.64	0.63	0.85	0.60	0.14	NS	NS	0.03
18:2	4.77	5.07	4.06	5.32	0.31	NS	0.01	NS
18:3	0.97	1.13	1.19	1.20	0.07	.05	NS	NS
Unsaturated	24.88	26.01	27.56	22.62	2.41	NS	NS	0.10
Saturated	75.12	73.99	74.44	77.38	4.40	NS	NS	0.10
Unsaturated:saturated	0.33	0.35	0.37	0.29	0.15	NS	NS	NS
Monounsaturated	18.50	19.17	17.47	15.50	2.90	NS	NS	NS
Polyunsaturated	6.38	6.83	6.10	7.12	0.90	NS	NS	NS

^an = 8 steers per treatment.

^b20 mg of Cu/kg DM as CuSO₄.

^cSoybean oil added at 4% of the diet DM.

^dFatty acids are presented as a percent of the fatty acids analyzed.

^eNS = Not significant ($P > 0.10$).

Table 8. Effects of copper and soybean oil (SBO) supplementation on fatty acid composition of longissimus muscle of finished steers^a

Fatty acid	Dietary treatment				SEM	Significance (<i>P</i> <)		
	No added Cu		Added Cu ^b			Cu	SBO	Cu × SBO
	No SBO	SBO ^c	No SBO	SBO ^c				
	g/100 g							
14:0	3.32	3.71	3.93	3.81	0.37	NS ^d	NS	NS
14:1	1.52	1.01	1.44	1.53	0.44	NS	NS	NS
15:0	0.72	.37	.68	.65	0.14	NS	NS	NS
16:0	28.89	29.99	31.79	29.30	1.6	NS	NS	NS
16:1 <i>cis</i>	3.9	3.11	4.61	3.50	0.34	0.12	0.01	NS
17:0	1.39	1.67	1.20	2.40	0.50	NS	NS	NS
18:0	12.89	12.69	10.79	12.49	0.74	0.10	0.11	NS
18:1 <i>trans</i>	6.00	8.51	6.11	7.89	0.29	0.12	0.02	NS
18:1 <i>cis</i>	35.01	31.50	32.41	30.80	1.50	NS	0.08	NS
18-conjugated dienes	0.20	0.29	0.23	0.35	0.08	NS	0.05	NS
18:2	5.51	6.60	6.30	7.1	0.67	NS	NS	NS
18:3	0.23	0.22	0.19	0.23	0.07	NS	NS	NS
22:0	0.13	0.09	0.19	0.23	0.04	.03	NS	NS
22:1	0.20	0.25	0.22	0.28	0.04	NS	NS	NS
Unsaturated	52.60	51.50	51.50	51.12	1.32	NS	NS	NS
Saturated	47.40	48.50	48.71	48.88	1.10	NS	NS	NS
Unsaturated:saturated	1.11	1.06	1.05	1.05	0.05	NS	NS	NS
Monounsaturated	46.70	44.38	44.80	44.79	1.5	NS	NS	NS
Polyunsaturated	5.94	7.11	6.68	7.70	.71	.14	NS	NS

^aFatty acids are presented as a percent of the fatty acids analyzed.

^b20 mg of Cu/kg DM as CuSO₄.

^cSoybean oil added at 4% of the diet DM.

^dNS = Not significant (*P* > 0.10).

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