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Effect of supplementation of two sources and two levels of copper on lipid metabolism in Nellore beef cattle

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ABSTRACT

This study was conducted with 35 Nellore beef cattle to determine the effect of supplementation of two levels and two copper sources (organic and inorganic) on metabolism of lipids and cholesterol of meat. The five treatments used were: Control: without copper supplementation, I10 or I40: 10 or 40 mg/kg DM (as Cu sulfate), O10 or O40: 10 or 40 mg/kg DM (as Cu proteinate). In general, the copper supplementation changed the fatty acid profile of meat ($p<0.05$), with a higher proportion of unsaturated fatty acids and reduction of saturated fatty acids. There was no effect of supplementation on blood cholesterol and triglycerides, however; in general, there was a reduction in cholesterol concentration in the *L. dorsi* ($p<0.05$) compared to the control treatment through the reduction ($p<0.05$) in the concentrations of GSH and GSH/GSSG ratio. The Cu supplementation did have an influence on metabolism of lipids. The production of healthier meat is beneficial to public health by reducing the risk of cardiovascular disease.

1. Introduction

The pursuit of a healthy diet has become a preoccupation for producers and the general public. Beef is rich in various nutrients that are frequently related to cardiovascular diseases due to elevated proportions of saturated fatty acids and cholesterol.

Various studies have suggested that copper (Cu) supplementation may decrease the cholesterol concentration in *Longissimus dorsi*, in addition to improving the fatty acid profile. Engle and Spears (2000b) evaluated Angus cattle with treatments of no supplementation, and with 10 mg Cu/kg of dry matter (DM) and 20 mg Cu/kg DM using $CuSO_4$ as the source of the copper. The cholesterol level in the muscle was observed to decrease with the use of the supplementation. There was also a significant increase in unsaturated fatty acids and a trend toward lower saturated fatty acids. The concentration of cholesterol serum in the animals supplemented with Cu was observed to decrease after the 56th day from the start of the study. Kim, Chao, and Allen (1992) reported that hepatic Cu indirectly regulates the cholesterol biosynthesis by the decrease of reduced glutathione (GSH) and the increase in the oxidized form (GSSG). GSH acts to stimulate the production of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA), which contributes to the organic synthesis of cholesterol. The effect of Cu supplementation on the production of healthier meat may benefit public health.

Minerals belong to a class of nutrients that have a significant contribution in a variety of biological functions. Because minerals interact with other nutrients as well as themselves, their study is complex, but is also essential to understand and solve problems that involve animal nutrition. Supplementation of Cu is generally required to overcome adverse effects caused by a deficiency of this element. Providing Cu in a readily available form that is not markedly influenced by Cu antagonists would be a logical approach to overcome these problems (Du, Hemken, Jackson, and Trammell, 1996). Improved availability of copper in the form of organic Cu complexes compared to the commonly used Cu salts (inorganic sources) has been suggested (Apgar and Kornegay, 1996). Thus, the use of Cu in organic form can improve the level of Cu in the body and hence improve the results obtained from the supplementation of Cu in the inorganic form, with respect to lipid metabolism and the reduction of cholesterol in meat.

The results of Cu supplementation in beef cattle vary, not only in relation to its chemical form, but also with respect to the animal breed, and because practically no research into the subject has been performed, a study of Cu supplementation for Nellore beef cattle presents an interesting opportunity. Thus, the objective of this study was to explore the effect of supplementation of two levels and two sources of Cu (inorganic and organic) on the metabolism of lipids and cholesterol in the meat of Nellore.

2. Material and methods

A total of 35 Nellore cattle, male with 30 months into the finishing phase were used for this study. The experiment lasted 84 days and

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was carried out at the Faculty of Animal Science and Food Engineering, University of São Paulo, at the Pirassununga Campus.

The animals were placed in individual stalls and underwent a period of 14 days of adaptation to the stalls and the diet before starting the experiment. During this period, they received a diet containing molybdenum (50 mg/kg) to reduce their copper level. After adaptation, a liver biopsy to verify the initial level of Cu was performed on the animals according to the technique described by Chapman, Cox, Haines, and Davis (1963).

Shortly thereafter, the cattle were divided into five groups totaling seven animals per treatment group. The treatment groups were 1) Co: basal diet without supplementation of copper, 2) I10: basal diet supplemented with 10 mg/kg of copper in the form of copper sulfate, 3) I40: basal diet supplemented with 40 mg/kg of copper in the form of copper sulfate, 4) O10: basal diet supplemented with 10 mg/kg of copper in the form of copper proteinate, and 5) O40: basal diet supplemented with 40 mg/kg copper in the form of copper proteinate. The basal diet contained 7 mg of Cu per kg DM. The diet supplied to the animals contained 30% of forage and 70% concentrate. The Cornell Net Carbohydrate and Protein System-CNCPS, version 5.0 was used to adjust the diets previously formulated according to NRC (1996) requirements for all nutrients, except copper.

Every 28 days blood samples were taken for analysis of total cholesterol and triglycerides. At the same time interval a Pie Medical Scanner 200 Vet ultrasound scanner with a 3.5 MHz, 18 cm linear transducer (Pie Medical Inc., Maastricht, The Netherlands) was used to measure the rib-eye area (REAU), the subcutaneous fat thickness (SFTU) between 12th and 13th ribs and the rump fat thickness (RFTU) in the upper third of the *Biceps femuris* muscle. Images were collected and interpreted by a technician certified by the Ultrasound Guidelines Council (UGC), using software CUP Lab (TM) (Walter and Associates, Inc., Ames, USA).

After a fasting period of 16 h following the end of the experiment, the animals were humanely slaughtered at the Abattoir-School of the Prefecture on the Pirassununga Campus. During the slaughter, liver samples were obtained from each animal and stored in liquid nitrogen for later analysis of GSH, GSSG and copper. After cleaning, the carcasses were kept in cold storage at 0 °C until completion of *rigor mortis*. After 24 h of chilling the left half of the carcasses were sawn between the 12th and 13th ribs to expose an area for the measurement of the subcutaneous fat thickness (SFTC) and the rib-eye area (REAC) using a ruled grid with a scale in cm^2. During boning, 5 g samples (in triplicate) were taken from 2.5 cm thick steaks cut from the *L. dorsi* muscle at the 13th rib towards head in the left half of the carcass, and these samples were immediately frozen in liquid nitrogen for analysis of cholesterol and fatty acids.

The concentrations of triglycerides and cholesterol in the plasma were determined by the enzymatic method using LABORLAB kits (LABORLAB Products for Laboratories LTDA, Guarulhos, São Paulo, Brazil). Analyses of cholesterol in the muscle were made by enzymatic method according to Saldanha, Mazalli, and Bragagnolo (2004). Muscle samples were crushed and divided into 2 g samples. After homogenization with KOH (50%) and ethyl alcohol in a water bath, distilled water was added and then hexane was used for phase separation. This extraction was repeated two more times and 3 ml of hexane extract was transferred to a glass tube, and then dried under N_2. The next steps were the addition of isopropanol, enzyme reagent, water bath and reading the absorbance. The copper in liver was analyzed by atomic absorption spectrophotometry. For the analysis, liver samples were thawed, had a separate fragment, taken always in the same region of the body, washed in distilled deionized water, dried in disposable absorbent paper and weighed with a precision scale. These determinations were made in the Laboratory of Mineral at Department of Animal Science, FZEA-USP. Measurements of GSH and GSSG in the liver and resulting GSH/GSSG ratio were made according to Tietze (1969). The total glutathione was measured after tissue homogenization in acid Sulphosalicylic 5% through recycling method in the presence of dinitrobenzoic acid (DTNB), NADPH and GR. The formation of trinitrobenzoic acid (TNB), which is a colored product absorbed in 412 nm, is proportional to the GSH (and GSSG) amount of the samples and was monitored spectrophotometrically for 3 min. The GSSG was determined by direct derivation of GSH when another sample of the same tissue was homogenized in the presence of N-ethylmaleimide (NEM 12.5 nM), followed by alkaline hydrolysis.

The fatty acid composition was performed in *Longissimus dorsi* muscle. The samples were removed and immediately frozen in liquid nitrogen for later analysis. Sample preparation (extraction and methylation) was performed in the laboratory of Animal Nutrition and Growth at the Department of Animal Production, ESALQ/USP, and qualitative determination of fatty acids was determined using gas chromatography in the Laboratory of Pesticide Residue at the Department of Entomology, ESALQ/USP. The extraction of lipids was performed using 5 g of muscle lyophilized sample, according to the methodology adapted from Hara and Radin (1978). The samples were placed in test tubes with 28 mL of hexane/isopropanol (3:2) v/v and homogenized (Polytron) for 1 min. After, they were vacuum filtered with filter paper, added a solution of sodium sulfate (67 g/L) to 50% of filtered volume and vortexed for 30 s. The supernatant layer was transferred to a tube containing 2 g of sodium sulfate, followed by insufflation of N^2 and allowed to rest for 30 min. Soon after, the liquid was transferred to vials of 10 mL, inflated N^2, sealed with a lid and kept at −20 °C until dry with N^2 for subsequent methylation. The extracted lipids were hydrolyzed and methylated according to the method described by Christie (1982), with modifications. Approximately 40 mg of lipids was transferred to a test tube and 2 mL of hexane was added, followed by 40 mL of methyl acetate, vortexed and added 40 mL of methylation solution (1.75 mL of methanol/0.4 mL of 5.4 mol/L of sodium methoxide). Then the mixture was further agitated by vortex for 2 min, followed by rest for 10 min, and added to 60 mL of reagent termination solution (1 g oxalic acid/30 mL diethyl ether), vortexed for 30 s, added 200 mg of calcium chloride and allowed to rest for 1 h. Subsequently they were centrifuged at 3200 rpm for 5 min at 5 °C and the supernatant was removed and placed in vials for later reading. The qualitative fatty acids determination was performed by gas chromatography in ThermoFinnigan chromatograph, model Trace with a flame ionization detector (FID) using fused silica capillary column of 100 m long, 0.25 mm diameter and 0.2 mm thick film (SP-2560 Supelco, Bellefonte, PA, USA). The carrier gas used was helium with a flow rate set at 1.2 mL/min. Was injected 1 mL of sample in split mode with split ratio of 1/21 in a temperature of 250 °C. The oven temperature was programmed to start at 70 °C and remained so for 4 min, raised to 170 °C to 13 °C/min and finally to 250 °C to 35 °C/min for 5 min. The detector temperature was 300 °C and the flow of gas was 450, 40 and 45 mL/min for synthetic air, hydrogen and nitrogen (makeup), respectively. The identification and recovery rate of fatty acid methyl esters were performed by comparison with retention times and fatty acid concentrations of a known standard (CRM-164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium).

The statistical analysis was performed using a completely randomized design with seven replicates per treatment. The data for REAC, SFTC, cholesterol, fatty acids and enzymes in the muscle or liver was analyzed with the SAS statistical software package (SAS Institute Inc., 1996) using PROC GLM for analysis of variance. PROC MIXED for SAS was used to analyze serum parameters taken every 28 days as well as the REAU, SFTU and RFTU data. These measures were analyzed as repeated measures in time. Averages were compared using contrasts and a 5% level of significance was adopted.

3. Results

The average of copper concentrations in the liver of cattle during the trial feedlot, are presented in Table 1.

Table 1
Average concentrations of copper (dry basis) in mg/kg liver of Nellore receiving the control diet or supplemented with different sources and levels of copper.

Variables (mg/kg)	Treatments					SEM	Orthogonal contrasts			
	Co	I10	I40	O10	O40		A	B	C	D
Liver copper	237.3	435.7	607.5	466.6	765.4	48.71	<**0.01**	**0.06**	**0.01**	<**0.01**

Co = control diet (without supplemental copper), I10 = supplementation of 10 mg Cu/kg DM in the form of copper sulfate, I40 = supplementation of 40 mg Cu/kg DM in the form of copper sulfate, O10 = supplementation of 10 mg Cu/kg DM in the form of copper proteinate, O40 = supplementation of 40 mg Cu/kg DM in the form of copper proteinate.
SEM = standard error of the mean.
A = control in comparison with others, B = Inorg in comparison with Org; C = I10 in comparison with I40; D = O10 in comparison with O40.

Table 2
Average values for subcutaneous fat thickness (SFTC) in mm, and rib-eye area (REAC) in cm^2, obtained during the boning of the *L. dorsi* muscle of the Nellore receiving the control diet or a diet supplemented with different sources and levels of copper.

Variables	Treatments					SEM	Orthogonal contrasts			
	Co	I10	I40	O10	O40		A	B	C	D
SFTC (mm)	5.00	5.00	5.57	5.14	5.86	0.41	0.40	0.61	0.33	0.23
REAC (cm^2)	82.00	82.00	83.57	82.00	78.86	3.25	0.91	0.47	0.73	0.50

Co = control diet (without supplemental copper), I10 = supplementation of 10 mg Cu/kg DM in the form of copper sulfate, I40 = supplementation of 40 mg Cu/kg DM in the form of copper sulfate, O10 = supplementation of 10 mg Cu/kg DM in the form of copper proteinate, O40 = supplementation of 40 mg Cu/kg DM in the form of copper proteinate.
SEM = standard error of the mean.
A = control in comparison with others, B = Inorg in comparison with Org; C = I10 in comparison with I40; D = O10 in comparison with O40.

The hepatic copper concentration was increased in animals supplemented with different sources and levels of this mineral.

The average values of fat thickness (mm) and rib-eye area (cm^2) of the cattle obtained using the measuring grid during boning and by ultrasound during the trial are presented in Tables 2 and 3. The values of rib eye area obtained by ultrasound were not very similar to those obtained in the carcass.

There was no significant effect ($p > 0.05$) on fat thickness and rib-eye area obtained by either ultrasound or during boning for the supplementation of copper from different sources and of varying levels. A quadratic effect for REAU and SFTU and linear effect for RFTU with the values increasing with the time of confinement was observed for measurements obtained by ultrasound during the experimental period. However, it may be noted that in relation to the absolute growth, REAU increased only 10.98%, while SFTU and RFTU increased 94.31% and 52.49%, respectively.

Table 4 shows the proportions of fatty acids in the *L. dorsi* muscle of the Nellore cattle for all treatments.

In general, the copper supplementation changed the fatty acid profile of meat ($p < 0.05$), with a higher proportion of unsaturated fatty acids over saturated fatty acids. In the case of fatty acids myristic (C14:0) and palmitic (C16:0), both considered hypercholesterolemic according to Farfan (1996), the supplementation of Cu regardless of the source and level, resulted in smaller percentages of these fatty acids in the beef cattle compared to the control. In the case of the stearic fatty acid (C18:0), supplementation with inorganic copper resulted in lower percentages compared to control. Cu supplementation, regardless of the source and level, increased the percentage of monounsaturated fatty acid oleic (C18:1c9), the unsaturated/saturated and monounsaturated/saturated ratios, and decreased the hyper/hypocholesterolemic ratio in the meat when compared to the control. There was no change in the percentage of fatty acids omega-3, omega-6, and in the omega-6/omega-3 ratio.

Average concentrations of triglycerides in the plasma and total cholesterol in the plasma and *L. dorsi* muscle of the cattle in relation to the treatment and time are shown in Table 5.

There was no effect of copper supplementation on the average concentrations of triglycerides and cholesterol in the plasma ($p > 0.05$). However, supplementation with O40 reduced the concentration of triglycerides by 23% compared to control. There was probably no significant effect due to the large variation in the data, observed by high coefficient of variation (38.35%). With regard to time, there was a quadratic effect for both the triglycerides variable ($y = 34.6483 + 0.8407x - 0.01477x^2$) and for cholesterol ($y = 188.36 + 2.1977x - 0.01810x^2$), with an increase in their concentrations in the plasma.

In the case of the *L. dorsi* muscle, the supplementation of copper regardless of source or level produces a reduction in the cholesterol concentration ($p < 0.05$) compared to the control treatment. The treatment with 40 mg/kg of inorganic Cu had lower muscular cholesterol concentration than the other treatments ($p < 0.05$). With respect to the control, supplementation with 40 mg/kg of Cu reduced the concentration of muscular cholesterol by approximately 11%.

4. Discussion

The liver is the central organ of metabolism of that mineral, so its concentration reflects the copper level being ingested by the organism (McDowell, 1992). This explains the values found for the treatments with copper supplementation. The analysis of liver samples is reliable to evaluate the copper status in the animal (Tokarnia, Döbereiner, Moraes, and Peixoto, 1999).

The lower growth for REAU in comparison to the fat measurements can be explained by the fact that the animals are at the stage where there is a decrease in muscle growth and a corresponding increase in body fat deposition.

With respect to copper supplementation, the results of this study were similar to a study by Engle and Spears (2000a), which also found no change in fat thickness of cattle following the termination of the supplementation of 20 mg Cu/kg DM. Other authors found a decrease in subcutaneous fat and increased rib-eye area with the supplementation of 40 mg Cu/kg DM (Engle and Spears, 2000b; Engle, Spears, Armstrong, and Odle, 2000a, Ward and Spears, 1997). One explanation for the reduction of fat thickness in these experiments was

Table 3
Average values for subcutaneous fat thickness (SFTU) and rump fat thickness (RFTU) in mm, and rib-eye area (REAU) in cm^2 obtained by means of ultrasound during the trial of Nellore fed the control diet or a diet supplemented with different sources and levels of copper.

Variables	Treatments					SEM	Orthogonal contrasts			
	Co	I10	I40	O10	O40		A	B	C	D
SFTU (mm)	4.76	4.82	4.91	4.59	4.93	0.34	0.95	0.78	0.51	0.73
RFTU (mm)	6.24	5.96	6.66	6.79	6.69	0.54	0.44	0.72	0.90	0.66
REAU (cm^2)	67.73	71.16	71.61	69.13	64.73	2.65	0.34	0.13	0.51	0.43

	Time (days)				Effect of time
	0	28	56	84	
SFTU (mm)	2.99 ± 0.23	4.92 ± 0.17	5.48 ± 0.23	5.81 ± 0.18	Q<0.01
RFTU (mm)	5.03 ± 0.27	6.28 ± 0.34	6.91 ± 0.38	7.67 ± 0.44	L<0.01
REAU (cm^2)	66.24 ± 1.32	67.88 ± 1.39	69.84 ± 1.32	73.51 ± 1.18	Q<0.01

Co = control diet (without supplemental copper), I10 = supplementation of 10 mg Cu/kg DM in the form of copper sulfate, I40 = supplementation of 40 mg Cu/kg DM in the form of copper sulfate, O10 = supplementation of 10 mg Cu/kg DM in the form of copper proteinate, O40 = supplementation of 40 mg Cu/kg DM in the form of copper proteinate.
SEM = standard error of the mean.
A = control in comparison with others, B = Inorg in comparison with Org; C = I10 in comparison with I40; D = O10 in comparison with O40.
L = linear effect; Q = quadratic effect.

Table 4
Fatty acid profile (%) of the *L. dorsi* muscle of Nellore receiving the control diet or the supplementation using different sources and levels of copper.

Fatty acids (%)	Treatments					SEM	Orthogonal contrasts			
	Co	I10	I40	O10	O40		A	B	C	D
C10:0 (Capric)[1]	0.08	0.07	0.07	0.07	0.08	0.01	0.18	0.78	0.50	0.36
C12:0 (Lauric)[1]	0.11	0.11	0.10	0.09	0.09	0.01	0.23	0.30	0.83	0.68
C14:0 (Myristic)[1]	4.35	3.53	3.00	3.17	3.50	0.24	<**0.01**	0.80	0.13	0.36
C14:1c9 (Myristoleic)[2,3]	0.92	1.02	0.80	0.75	0.89	0.10	0.62	0.40	0.14	0.34
C15:0 (Decapentaenoic)[1]	0.38	0.37	0.29	0.34	0.36	0.03	0.23	0.54	0.13	0.54
C16:0 (Palmitic)[1]	27.54	25.69	24.64	24.49	24.96	0.61	<**0.01**	0.47	0.23	0.59
C16:1c9 (Palmitoleic)[2,3]	2.64	3.38	2.88	2.78	2.97	0.24	0.19	0.29	0.15	0.59
C17:0 (Margaric)[1]	0.88	0.88	0.90	0.84	0.86	0.08	0.88	0.58	0.80	0.88
C17:1[2,3]	0.76	0.80	0.70	0.75	0.78	0.06	0.94	0.85	0.24	0.65
C18:0 (Stearic)[1]	16.10	13.80	13.03	14.76	15.09	0.55	<**0.01**	**0.01**	0.33	0.67
C18:1 c9 (Oleic)[2,3]	35.84	40.67	39.75	39.86	40.49	1.15	<**0.01**	0.98	0.58	0.70
C18:1 c11[2,3]	1.50	1.41	1.38	1.41	1.35	0.09	0.28	0.89	0.84	0.66
C18:1 c12[2,3,6]	0.22	0.18	0.22	0.23	0.19	0.02	0.45	0.60	0.21	0.13
C18:1 c13[2,3]	0.38	0.46	0.36	0.37	0.43	0.04	0.58	0.70	0.08	0.30
C18:1 c15[2,3,5]	0.07	0.09	0.07	0.07	0.09	0.01	0.45	0.89	0.07	0.09
C18:1 t16[2,3]	0.17	0.17	0.16	0.17	0.19	0.01	0.67	0.37	0.34	0.25
C18:2c9c12 (Linoleic)[2,4,6]	2.88	2.91	4.43	3.78	3.28	0.60	0.30	0.82	0.08	0.57
C18:2c9 t11 (CLA)[2,4]	0.44	0.48	0.48	0.44	0.50	0.04	0.43	0.69	0.99	0.30
C18:2 t11 c15[2,4,5]	0.04	0.04	0.12	0.09	0.03	0.03	0.34	0.47	0.08	0.24
C18:3n3 (Linolenic)[2,4,5]	0.32	0.28	0.40	0.42	0.31	0.07	0.66	0.76	0.26	0.31
C18:3n6 (Linolenic)[2,4,6]	0.14	0.10	0.12	0.10	0.11	0.02	0.07	0.63	0.39	0.65
C20:1 (Eicosanoic)[2,3]	0.16	0.18	0.16	0.16	0.17	0.02	0.65	0.90	0.56	0.70
C20:4 (Arachidonic)[2,4]	0.71	0.60	1.43	1.48	0.83	0.35	0.35	0.68	0.10	0.20
C20:5 (EPA)[2,4]	0.33	0.16	0.38	0.37	0.21	0.11	0.69	0.89	0.16	0.31
C22:2[2,4]	0.12	0.07	0.15	0.12	0.09	0.03	0.60	0.93	0.07	0.39
C22:5[2,4]	0.50	0.34	0.75	0.76	0.46	0.19	0.72	0.73	0.13	0.26
C22:6[2,4]	0.05	0.03	0.12	0.08	0.06	0.03	0.46	0.87	0.07	0.59
Saturated	50.19	45.10	42.79	44.35	45.50	1.23	<**0.01**	0.43	0.19	0.51
Unsaturated	49.81	54.90	57.21	55.65	54.50	1.23	<**0.01**	0.43	0.19	0.51
Monounsaturated	44.20	49.84	48.75	47.94	48.57	1.25	<**0.01**	0.41	0.54	0.72
Polyunsaturated	5.61	5.06	8.46	7.71	5.93	1.25	0.41	0.96	0.07	0.32
Unsaturated/saturated	0.99	1.22	1.34	1.27	1.23	0.07	<**0.01**	0.57	0.22	0.67
Mono/saturated	0.88	1.11	1.14	1.09	1.08	0.05	<**0.01**	0.44	0.67	0.94
Poly/saturated	0.11	0.11	0.20	0.17	0.14	0.03	0.22	0.96	0.07	0.46
Omega-3	0.43	0.41	0.59	0.57	0.43	0.07	0.39	0.98	0.09	0.18
Omega-6	3.26	3.20	4.78	4.12	3.50	0.60	0.34	0.82	0.08	0.54
Omega-6/omega-3	7.99	7.85	8.48	7.92	7.85	0.96	0.98	0.77	0.64	0.96
Hyper/hipo[7]	0.64	0.53	0.48	0.50	0.53	0.02	<**0.01**	0.83	0.16	0.41

Co = control diet (without supplemental copper), I10 = supplementation of 10 mg Cu/kg DM in the form of copper sulfate, I40 = supplementation of 40 mg Cu/kg DM in the form of copper sulfate, O10 = supplementation of 10 mg Cu/kg DM in the form of copper proteinate, O40 = supplementation of 40 mg Cu/kg DM in the form of copper proteinate.
SEM = standard error of the mean.
A = control in comparison with others, B = Inorg in comparison with Org; C = I10 in comparison with I40; D = O10 in comparison with O40.
[1]Saturated; [2]Unsaturated; [3]Monounsaturated; [4]Polyunsaturated; [5]Omega-3; [6]Omega-6; [7]Hypercholesterolemic (C14:0 + C16:0)/hypocholesterolemic (monounsaturated + polyunsaturated); (Silva, 2005).

due to the decreased performance of the animals supplemented with high doses of Cu. Another explanation may be based on the relationship of Cu with the fatty acid synthase enzyme. Konjufca, Pesti, and Bakalli (1997) in a study of birds found that increased levels of Cu caused a reduction of this enzyme activity, and consequently the synthesis of fat, decreasing the thickness of fat in the meat. In this

Table 5
Average concentrations of triglycerides and total cholesterol in the plasma in mg/deciliter and cholesterol (mg/100 g of meat) in the *L. dorsi* muscle of Nellore fed the control diet or the diets supplemented from different sources and levels of Cu during the experimental confinement.

Variables	Treatments					SEM	Orthogonal contrasts			
	Co	I10	I40	O10	O40		A	B	C	D
Triglycerides (mg/deciliter)	45.94	39.44	38.89	41.45	35.37	2.54	0.97	0.59	0.79	0.26
Cholest. plasm (mg/deciliter)	227.2	228.1	233.9	236.6	220.9	11.79	0.91	0.35	0.87	0.71
Cholesterol muscle (mg/100 g meat)	105.4[a]	98.5[b]	94.1[c]	100.5[b]	97.8[b]	1.12	<**0.01**	**0.02**	**0.01**	0.10

	Time (days)				Effect of time
	0	28	56	84	
Triglycerides (mg/deciliter)	34.9 ± 1.63	36.9 ± 3.24	42.3 ± 2.78	46.8 ± 3.29	Q<0.01
Cholest. plasma (mg/deciliter)	187.3 ± 4.52	236.6 ± 6.20	245.4 ± 9.15	248.0 ± 7.24	Q<0.01

Co = control diet (without supplemental copper), I10 = supplementation of 10 mg Cu/kg DM in the form of copper sulfate, I40 = supplementation of 40 mg Cu/kg DM in the form of copper sulfate, O10 = supplementation of 10 mg Cu/kg DM in the form of copper proteinate, O40 = supplementation of 40 mg Cu/kg DM in the form of copper proteinate.
SEM = standard error of the mean.
A = control in comparison with others, B = Inorg in comparison with Org; C = I10 in comparison with I40; D = O10 in comparison with O40.
Averages followed by different letters in rows differ ($p<0.05$), using the analysis of contrasts.
Q = quadratic effect.

experiment, the activity of the fatty acid synthase enzyme was not measured, however, this study of Konjufca et al. (1997) used much higher levels of Cu (180 mg/kg DM), which may explain the absence of similar results in the current study.

According to Wood et al. (2003), the omega-6/omega-3 ratio is very important because it is associated with coronary heart disease and cancer. The omega-6/omega-3 ratio recommended a diet that should be less than 4.5. The value found was 8.0 in the meat, but this is only a particular food, does not represent the total diet. This ratio increases as the animals stay longer in confinement, or get fatter, since the proportion of fat in the lipid droplets increases in relation to the proportion of fat in the membrane, i.e., the more the fat is deposited the worse the ratio becomes (Duckett, cited by Silva, 2005). In this study, the animals deposited a significant amount of fat, as can be seen by levels of SFTU and RFTU, and this may explain for the higher omega-6/omega-3 ratio.

On average, carcasses of beef contain 49% of saturated fatty acids, 45% monounsaturated and 6% polyunsaturated (Duckett, Wagner, Yates, Dolezal, and May, 1993), which are close to the values found for the control treatment in this study. Supplementation with Cu was effective in reducing the percentage of saturated fatty acids and increasing the unsaturated, especially monounsaturated fats. Other authors working with Cu supplementation in similar levels to this study also observed alterations in the profile of fatty acid with a reduction of saturated fatty acids and increased unsaturated fatty acids (Engle and Spears, 2000b; Engle and Spears, 2001; Engle et al., 2000a). According to these authors, this may occur due to a drop in ruminal biohydrogenation promoted by the Cu, and the consequential increase in intestinal absorption of unsaturated fatty acids. According to Berchielli, Pires, and Oliveira (2006), the fatty acid composition in meat is the result of lipogenesis in adipose tissue and also of dietary lipids and subsequent ruminal biohydrogenation. Engle, Spears, and Edens (2000b) explained that the high reduction potential of Cu in the rumen may decrease reducing equivalents in the form of NADH and NADPH, which interferes with microbial biohydrogenation of unsaturated fatty acids. This may explain the change in fatty acid composition with the addition of Cu, i.e. the influence of this mineral in ruminal biohydrogenation.

Some authors have found a decrease in the levels of triglycerides and/or cholesterol in the blood with the supplementation of Cu in various animal species (Alarcon-Corredor et al., 2000; Engle and Spears, 2000b; Engle et al., 2000b), which is an observation that differs from the results of the current study. However, Engle and Spears (2001) in a study of Simmental cattle using supplementations with different levels of Cu, no changes were found in plasma and muscle cholesterol with the supplementation of Cu.

In a study by Engle et al. (2000a) of cattle through growing and finishing, the supplementation of Cu did not alter the serum cholesterol levels during the growth phase, and during the finishing phase the serum cholesterol was not affected until day 56, but a decrease was observed on days 84, 96 and 116 in the cattle supplemented with copper in comparison to the control group. In this study, Cu supplementation was performed for only 84 days, so this may be one explanation for the lack of a significant effect. In another study of cattle, Del Claro (2007) found no change in the blood cholesterol concentration with the supplementation of Cu, as is the case with the current study. According to the author, the total cholesterol is mostly composed of LDL, which has a long half-life, i.e. the effect of reduced synthesis may have already occur by the 84th day, however, has still not appeared as a function of the long half-life of LDL.

Many studies have suggested that copper supplementation may affect the cholesterol metabolism in both ruminants and non-ruminants. Similar to this study, a study of cattle by Engle and Spears (2000b) and Engle et al. (2000a) found that the addition of different levels of Cu in the diet resulted in a reduction in cholesterol concentration in the *L. dorsi* muscle for the treatments with the supplementation. With respect to non-ruminants, Konjufca et al. (1997) found a reduction in cholesterol levels in the breast and thighs of broilers supplemented with organic and inorganic Cu. Bakalli, Pesti, Ragland, and Konjufca (1995), Pesti and Bakalli (1996) and Skrivan, Sevcikova, and Tumova (2002) found that a reduction of cholesterol in chicken muscle was the main effect of supplementation with Cu. According to Klevay (1973), rats fed diets deficient in Cu developed hypercholesterolemia.

Kim et al. (1992) found that Cu deficiency in rats caused hypercholesterolemia by increasing hepatic GSH with increasing HMG-CoA reductase activity. According to these authors, Cu-deficient animals had increased hepatic GSH, inhibition of the peptide by a specific inhibitor (BSO), reduced HMG-CoA reductase activity, and reduction of cholesterol synthesis.

Recent studies have shown that the thiol/disulfide ratio of cells may influence the activity of several key enzymes that require thiol groups for activity (Gilbert, 1984; Gilbert, 1990). One of these enzymes is HMG-CoA reductase, whose activity is considered the step in controlling the rate of cholesterol synthesis (Murray, Granner, Mayes, and Rodwell, 1998; Yount, McNamara, and Al-Othman, 1990). In vitro studies have shown that this enzyme requires thiol for its activity; GSH being the main one (Roitelman and Shechter, 1984), and that small concentrations of disulfides such as GSSG decreased the enzyme activity (Gilbert, 1990; Ziegler, 1985). Thus, the most accepted hypothesis is that Cu indirectly regulates hepatic cholesterol biosynthesis by decreasing GSH and increasing the GSSG. GSH would stimulate the production of HMG-CoA, therefore, if the Cu supplementation decreases the cellular concentration of GSH, then it decreases cholesterol synthesis (Bakalli et al., 1995).

In the current study, there was a reduction of GSH and the GSH/GSSG ratio with the supplementation of copper, as can be seen in Table 6. Therefore, a drop in the enzyme HMG-CoA reductase may have occurred. The enzyme activity was not measured; however, the cited relationship between it and the metabolism of GSH may explain the results, i.e. the reduction of cholesterol levels in the *L. dorsi* muscle in treatments with Cu.

Table 6
Average concentrations of GSH and GSSG in μmol/g of liver and GSH/GSSG ratio of Nellore cattle fed the control diet or the diet supplemented with different sources and levels of copper.

Variables (μmol/g liver)	Treatments					SEM	Orthogonal contrasts			
	Co	I10	I40	O10	O40		A	B	C	D
GSH	1.84	1.58	1.36	1.44	1.31	0.14	**0.01**	0.48	0.27	0.50
GSSG	0.012	0.014	0.017	0.018	0.014	0.002	0.08	0.97	0.18	0.11
GSH/GSSG	158.1	124.7	80.8	90.8	100.9	14.76	<**0.01**	0.65	**0.04**	0.63

Co = control diet (without supplemental copper), I10 = supplementation of 10 mg Cu/kg DM in the form of copper sulfate, I40 = supplementation of 40 mg Cu/kg DM in the form of copper sulfate, O10 = supplementation of 10 mg Cu/kg DM in the form of copper proteinate, O40 = supplementation of 40 mg Cu/kg DM in the form of copper proteinate.
SEM = standard error of the mean.
A = control in comparison with others, B = Inorg in comparison with Org; C = I10 in comparison with I40; D = O10 in comparison with O40.

5. Conclusion

Copper (Cu) supplementation influenced the metabolism of lipids, increasing the level of unsaturated fatty acids over saturated and reducing the levels of cholesterol in the *L. dorsi* muscle due to the change in the GSH/GSSG ratio. The production of healthier meat is beneficial to public health by reducing the risk of cardiovascular disease.

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