PE8.26 Effects of copper source and level on the lipid oxidation and colour in meat of Nellore cattle 262.00

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***Abstract*—Lipid oxidation can be affected by the diet and by the presence of antioxidants or pro-oxidants. Some copper-dependent antioxidant enzymes can inhibit lipid oxidation and increase the stability of the meat. Thus, thirty five Nellore bulls were used to determine the effect of supplementation of two levels and two sources of copper (inorganic and organic) on the oxidative stability and color of meat. Treatments were: 1) Control (C) - without supplemental Cu; 2) Cu (I10) - 10 mg/kg DM (as Cu sulfate); 3) Cu (I40) - 40mg/kg DM (as Cu sulfate); 4) Cu (O10) - 10 mg/kg DM (as Cu proteinate); 5) Cu (O40)- 40 mg/Kg DM (as Cu proteinate). The lipid oxidation was determined in meat samples submitted to 0, 1, 2 and 3 days of Display Life (DL), 0, 3, 6 and 9 days of Modified Atmosphere (ATM) and 0, 7 and 14 days of Vacuum Packing (VC), using the technique of Thiobarbituric Acid Reactive Substances (TBARS). The surface colour of meat was analyzed using a colorimeter (MiniScan XE model, Hunterlab), using the CIELab scale (L*, a*, b*). Statistical analyses of data were performed by ANOVA for repeated measures within a completely randomized design, using the PROC MIXED procedure of SAS. There was a significant effect (p <0.05) of treatment and time on lipid oxidation of Nellore cattle meat during periods of DL and ATM. For samples packaged in vacuum, time was the only significant effect (p <0.05) observed on lipid oxidation. In general, there was an increase in TBARS as a function of storage time for DL, ATM and VC samples. For the DL and ATM samples, the values of TBARS were lower for animals supplemented with 40mg Cu/kg DM in relation to those that received control diet. There was a significant effect (p <0.05) of time on Hunter color values (L, a * b *) of DL and ATM samples. There was no effect of treatments on these variables. Dietary supplementation with Cu may improve the oxidative stability of beef, showing the antioxidant effect of this mineral, but does not affect the general appearance of meat.**

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***Index Terms*—antioxidants, meat, minerals. TBARs.**

I. INTRODUCTION

The minerals participate of many biological functions and interact with other nutrients and also among themselves. Thus, their study becomes complex, however essential to understanding and solve problems involving animal nutrition. In Brazil, there is proven copper (Cu) deficiency in most regions, and the fact that Cu has great importance for cattle, the study of its sources is crucial, since its supplementation is essential. Furthermore, it has been disclosed that the organic Cu has greater bioavailability than inorganic sources, by not interacting with other elements and perhaps to be absorbed by different metabolic routes.
The lipid oxidation can be defined as a cascade of biochemical events, resultant from the action of free radicals on lipids from cell membranes, generating mainly radical Alcoxil and Peroxil. The lipid in meat products oxidizes easily, causing the development of unpleasant odor and flavor, known as "warmed-over-flavor" [1].

The lipid oxidation can be affected by the diet and by the presence of antioxidants or pro-oxidants [2]. Some studies have shown that depending on the form in which copper is found, it can have a pro or an antioxidant effect [3]. When in ionic form, inorganic copper is a pro-oxidant element and can stimulate lipid oxidation. Furthermore, the organic copper, as it is encountered in a chelate form, would not have deleterious effects. Thus, it is interesting to study the possible effects of this mineral in meat from cattle. Some copper-dependent antioxidant enzymes can

inhibit lipid oxidation and increase the stability of the meat, which can be measured by the technique of TBARS (Thiobarbituric Acid Reactive Substances). Thus, the objective of this research was to determine the effect of supplementation of two copper levels and sources (inorganic and organic) on the oxidative stability and color of meat from Nellore cattle.

II. MATERIALS AND METHODS

A. Local

The experiment was conducted in the Faculty of Animal Science and Food Engineering of University of São Paulo, Pirassununga Campus, State of São Paulo, Brazil, for a period of 112 days.

B. Animals

Thirty-five Nelore bulls with approximately 2.5 years old in the finishing phase were used in this experiment. The animals were placed in individual pens.

C. Treatments

On arrival, the animals went through in a period of adaptation to the pens and during this period they were fed a diet with molybdenum (50 mg / kg) to reduce their copper status. After adaptation, liver biopsy was performed in animals, according to the procedure previously described [4], to verify their initial Cu status. Then, animals were randomly assigned in five groups, in a total of seven animals per treatment. The treatments were: 1) Control (C): basal diet without additional copper supplementation; 2) Cu (I10): basal diet with supplementation of 10 mg Cu/kg DM as copper sulfate; 3) Cu (I40): basal diet with supplementation of 40 mg Cu/kg DM as copper sulfate; 4) Cu (O10): basal diet with supplementation of 10 mg Cu/kg DM as copper proteinate; 5) Cu (O40): basal diet with supplementation of 40 mg Cu/kg DM as copper proteinate. All animals received diet containing 30% corn silage and 70% concentrate. The program Cornell Net Carbohydrates and Protein System-CNCPS was used to adjust the diets previously formulated into accordance with the requirements of the NRC [5] for all nutrients except Cu.

D. Experimental procedure

The slaughter was performed following humanity standard procedures at a local slaughterhouse. The captive bolt method was used to stun the animals. Carcasses were split, weighed and then chilled at 0-3ºC before processing on the following day after slaughter. At 24 hours *post* mortem, *Longissimus dorsi* (LD) muscle (13^{th} through the 10^{th} rib) from right carcass were removed, cut into 2.5 cm thick steaks.

From one steak, the surface colour was measured and then samples of 5g (in triplicate) were collected and immediately frozen using liquid Nitrogen (N_2) for posterior TBARS analysis (Day 0, used as reference value).

The steaks for the analysis of Display Life (DL) and Modified Atmosphere (ATM) were placed on expanded polystyrene trays and covered with poly (vinyl chloride) films.

The trays for the analysis of DP were placed in refrigerated display counter (model LX 125 vega, mark Auden) at 4^oC where they remained for up to 3 days. In each day, 7 trays from each treatment were removed from the refrigerated display counter, surface colour was measured and samples of 5g (in triplicate) for TBARS analysis were collected and frozen in N_2 (DL Days 1, 2 and 3).

The trays for the analysis of ATM were placed in a second masterpack - MP (78.5 cm x 48.5 cm. 0.35m^2; Cryovac) with high gas barrier property, 5 trays per masterpack, containing 75% O_2:25% CO_2 gas composition. After sealing, the atmosphere composition inside the MP was checked using a Dansensor gas analyzer (CheckPoint O2/CO2). No significant variation on the mixture was found during the storage. The MPs were stored in a refrigerated chamber (2.0±1.0ºC) for 9 days. In the 1^{st}; 3^{rd}; and 9^{th} storage days, 7 MP packs were removed from the refrigerated chamber and meat surface colour was measured and samples of 5g (in triplicate) for TBARS analysis were collected and frozen in N_2 (ATM Days 3, 6 and 9).

The steaks for the analysis of maturation were Vacuum Packing (VC) and stored in a refrigerated chamber (2.0±1.0ºC) for up to 14 days. At 7 and 14 days, samples from 5 g of meat (in triplicate) were collected and immediately frozen in N2 for TBARS analysis (VC Days 7 and 14).

E. Analytical Procedures

The method of 2-thiobarbituric acid reactive substances (TBARS) in meat was determined according to the procedure previously described in the literature [6].

The surface colour of meat was analyzed using a colorimeter (MiniScan XE model, Hunterlab), using the CIELab scale (L*, a*, b*).

F. Statistical Analysis

Statistical analyses of data were performed by ANOVA for repeated measures within a completely randomized design, with two-factor analysis in factorial scheme using the PROC MIXED procedure of SAS [7], with 5% significance.

III. RESULTS AND DISCUSSION

There was a significant effect ($p < 0.05$) of treatment and time on lipid oxidation of Nellore cattle meat during periods of Display Life and Modified Atmosphere. For samples packaged in vacuum (VC), time was the only significant effect ($p < 0.05$) observed on lipid oxidation. Effects of time and treatments on lipid oxidation were presented in Table 1 and Figure 1, respectively.

Table 1. TBARS (mg malonaldehyde/kg) in meat of Nellore bulls submitted to 0, 1, 2 and 3 days of Display Life (DL), 0, 3, 6 and 9 days of Modified Atmosphere (ATM) and 0, 7 and 14 days of Vacuum Packing (VC)

TBARS	Time (Days)				*SE*
	0d	*1d*	*2d*	*3d*	
DL	0.1283^{c}	0.1483^{b}	0.1524^{b}	0.1627^{a}	0.003
	0d	*3d*	*6d*	*9d*	
ATM	0.1283^{c}	0.1703^{b}	0.1845^{a}	0.1932^{a}	0.004
	0d	*7d*	*14d*		
VC	0.1283^{c}	0.1455^{b}	0.1554^{a}		0.004

SE = Standard error;
Averages followed by equal letters in the rows do not differ ($p>0,05$).

In general, there was an increase in TBARS as a function of storage time (d 0, 1, 2 and 3) for DL samples. The TBARS values obtained in animals receiving diet with 40ppm copper, independent the copper source (organic or inorganic), were lower when compared to control animals.

For the Modified Atmosphere samples, it was also observed that TBARS increased as a function of storage time (d 0, 3, 6 and 9), and TBARS values were lower for animals supplemented with copper in relation to those that received the control diet. Within the 40ppm level, there was a difference between sources, and the animals supplemented with inorganic source had lower TBARS than those supplemented with the organic source. For vacuum packaged samples, TBARS increased in the period of maturation (d 0, 7 and 14).

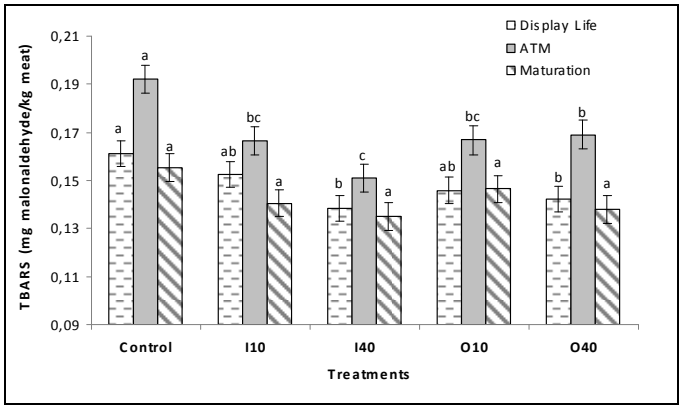

Figure 1. Lipid oxidation in *L. dorsi* muscle of Nellore bulls receiving a control diet (without copper supplementation), I10 (10mg Cu/kg DM as Cu sulfate), I40 (40mg Cu/kg DM as Cu sulfate), O10 (10mg Cu/kg DM as Cu proteinate) and O40 (40mg Cu/kg DM as Cu proteinate).

With respect to storage time, it is expected to exist an increase in TBARS values, since, regardless the presence of oxygen, there are metabolic processes that continuously generate free radicals that may cause lipid oxidation. The reduction in TBARS values in meat from bulls supplemented with copper for the samples submitted in Display Life (I40 and O40) and Modified Atmosphere (I10, I40, O10 and O40), in relation to control animals, suggest that this mineral exerts an antioxidant effect. There was no pro-oxidant effect of inorganic copper.

TBARS values were higher for the ATM samples than for DL and VC samples, because of a higher concentration of oxygen in ATM packing. It's already known that high O2 concentrations may negatively impact the oxidative stability of muscle lipids, causes the rapid development of rancidity meat, and consequently affect meat quality attributes [8, 9]. The lack of significant effects of treatments found in this study, for samples packed under vacuum (VC), may be due to the absence of oxygen, not being possible to observe antioxidant activity of copper during the 14 days of storage.

There were no studies so far evaluating the relationships between copper supplementation and lipid oxidation in cattle. In an experiment in which the chemical composition and the lipid oxidation of *L. dorsi* muscle of pigs were evaluated, it was observed that copper supplementation did not affect the susceptibility to lipid oxidation [2]. Some authors investigated the effect of supplementation of different Cu levels and vitamin E on oxidative and antioxidative status of growing pigs and found no pro-oxidant effect of Cu. The increase of TBARS in liver was reduced by the addition of vitamin E and Cu in the diet, indicating

an antioxidative effect of both nutrients [10]. In order to study the effect of α-tocopherol and $CuSO_4$ on susceptibility of meat to oxidation in pigs other authors found lower TBARS values in the groups supplemented with Cu and α-tocopherol, when compared to the control [11].

There was a significant effect ($p < 0.05$) of time on the Hunter color values (L*, a * b *) of meat in the Display Life and in Modified Atmosphere storage. There was no effect of treatments on these variables. Mean values of L*, a * and b * of meat pooled by treatments and by storage times are presented in Table 2 and 3, respectively.

Table 2. Mean Hunter color values (L*, a * b *) measured in meat from Nellore bulls submitted to Display Life (DL) or Modified Atmosphere (ATM).

	Treatments					*SE*
	C	*I10*	*I40*	*O10*	*O40*	
L*						
DL	38.8[a]	40.2[a]	40.6[a]	39.6[a]	39.7[a]	0.49
ATM	41.1[a]	42.6[a]	43.0[a]	41.9[a]	42.6[a]	0.47
a*						
DL	19.3[a]	20.1[a]	20.3[a]	19.7[a]	20.5[a]	0.43
ATM	21.6[a]	21.6[a]	22.1[a]	21.9[a]	21.2[a]	0.44
b*						
DL	15.8[a]	16.8[a]	17.0[a]	16.1[a]	16.5[a]	0.30
ATM	16.6[a]	17.1[a]	17.4[a]	16.9[a]	17.1[a]	0.26

C = Control diet (without copper supplementation); I10 = 10mg Cu/kg DM (as Cu sulfate); I40 = 40mg Cu/kg DM (as Cu sulfate); O10 = 10 mg Cu/kg DM (as Cu proteinate); O40 = 10mg Cu/kg DM (as Cu proteinate);
SE = Standard error;
Averages followed by equal letters in the rows do not differ ($p>0.05$).

In general, L *, a * b * determined in this study according to the times of Display Life and Modified Atmosphere storage had similar behavior (Table 3). Although the values determined in meat samples of ATM were higher than that determined during the DL, this difference can be attributed to the high concentration of oxygen that promotes greater color stability in meat [12].

Table 3. Mean Hunter color values (L, a * b *) measured in meat from Nellore bulls submitted to 0, 1, 2 and 3 days of Display Life (DL) and 0, 3, 6 and 9 days of Modified Atmosphere (ATM).

	Time				*SE*
DL	*0d*	*1d*	*2d*	*3d*	
L*	38.85[b]	40.50[a]	40.69[a]	39.17[b]	0.30
a*	16.22[c]	21.91[a]	21.50[a]	20.32[b]	0.31
b*	13.99[d]	18.74[a]	17.17[b]	15.88[c]	0.20
ATM	*0d*	*3d*	*6d*	*9d*	
L*	38.85[c]	42.70[b]	43.18[b]	44.27[a]	0.28
a*	16.22[c]	24.35[a]	23.95[a]	22.95[b]	0.27
b*	13.99[c]	19.28[a]	17.55[b]	17.28[b]	0.16

SE = Standard error;
Averages followed by equal letters in the rows do not differ ($p>0.05$).

IV. Conclusion

Dietary supplementation with copper may improve the oxidative stability of beef, showing the antioxidant effect of this mineral, but does not affect the general appearance of meat. Dietary supplementation with 40mg Cu/kg DM in inorganic form was more effective in reducing lipid oxidation in beef of Nellore bulls.

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