PROTEIN OXIDATION AND COLOR STABILITY IN MEAT AGED UNDER AEROBIC CONDITIONS FROM BULL AND STEER

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Abstract – The aim of this work was to evaluate the protein oxidation and color stability in beef aged under aerobiosis condition. Bulls and steers were used as models, since they accumulate fat in carcass differently. Twelve male cattle (6 bulls and 6 steers) of the Nellore breed, confined for 120 days, were used. After 48 h post-mortem, steaks were collected from *Longissimus dorsi* (*LD*) muscle and aged for 1, 3, 5, 7, and 9 days under aerobiosis. Analyses of pH, thiol groups, and metmyoglobin were carried out. The results showed that the castration of male cattle had only effect ($P < 0.05$) on the pH values, where the steaks from bulls presented higher ($P < 0.05$) values with regard to the steaks from steers. The thiol groups and the metmyoglobin were only influenced ($P < 0.05$) by the time of aging. A relationship between those two variables was observed at the last time of aging, where a decrease ($P < 0.05$) of thiol groups resulted in an increase ($P < 0.05$) of metmyoglobin. Thus, it was concluded that the castration does not affect thiol groups and metmyoglobin in *LD* muscle from Nellore cattle. Also, the aging of the meat under aerobiosis condition results in protein oxidation (reduction in thiol groups) and in appearance of the metmyoglobin.

Key Words – castration, oxygen, protein oxidation

I. INTRODUCTION

The protein oxidation is one of the most important indicators of meat deterioration [1], being responsible for the fragmentation, aggregation, and solubility decrease of proteins through the modifications of amino acids [2].

Other aspect of meat quality influenced by the protein oxidation would be the discoloration [3]. The meat coloration has been a decisive factor during the purchase and is related to concentration and chemical state of the myoglobin. Oxidation of the oxymyoglobin results into the metmyoglobin, pigment of brown color, which is undesirable by the consumers [4].

A longer time of aging, availability of oxygen, and presence of lipids collaborate to increase metmyoglobin and to decrease thiol groups [5]. Hence, the purpose of this work was to evaluate the effect of aging times on the protein oxidation and color stability in meat under aerobiosis conditions from bulls and steers, since the castration has increased the amount of fat in the carcasses [6,7].

II. MATERIALS AND METHODS

A. Animals

For this experiment, twelve male cattle (6 bulls and 6 steers) of the Nellore breed (*Bos indicus*) with 25 months of age were used. All the animals were confined for 120 days with a common diet composed of sugarcane bagasse, corn grain, soybean meal, soybean hull, urea, and mineral trace. The animals were slaughtered at an average weight of 523 ± 26 kg.

B. Muscle

After 48 hours post-mortem, meat samples from the *Longissimus dorsi* (*LD*) muscle were cut in steaks with 2.0-cm thick, which were wrapped in polyvinyl chloride (PVC) film and aged for 1, 3, 5, 7, and 9 days at 2 ± 2 ºC.

The pH values were taken at three different points of the steaks by inserting a pH meter (Hanna Instruments, model HI99163) into a small incision.

Thiol groups were determined as described before [8]. An extraction of proteins in 5% SDS in 0.1 M of Tris buffer (pH=8.0) was done using an Ultra Turrax. The homogenates were placed in a water bath at 80ºC for 30 minutes and filtered. The protein concentration of the filtrate was determined by measuring absorbance at 280 nm using BSA as a standard curve. The filtrates were diluted to a concentration of 1.5 mg/mL with the buffer used for homogenization and assayed according to previous work [9], using 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) in the reaction. Thiol content was measured by absorbance at 412 nm and expressed in nmol thiol/mg of protein.

In turn, the metmyoglobin were carried out as described previously [10]. The meat samples were homogenized in 20 mL phosphate buffer Na^+/K^+ 0.04 mol/L (pH 6.8) on 14,000 rpm for 20 seconds using an Ultra Turrax. After 1 hour under ice, the homogenate was centrifuged at 10,000 g for 30 minutes at 10ºC. The supernatant was filtered by a Whatman #1 filter paper. The volume was completed up to 25 mL and was again filtered with Millipore membrane (0.25 μm). The filtrate was read at absorbance of 525 (A^{525}), 503 (A^{503}), 557 (A^{557}), and 582 (A^{582}) nm in a spectrophotometer. The formula described by Tang *et al.* [11] was used to calculate the metmyoglobin percentage: $-0.159 \times (A^{582}/A^{525}) - 0.085 \times (A^{557}/A^{525}) + 1.262 \times (A^{503}/A^{525}) - 0.520$.

C. Statistical Analysis

A completely randomized design with a 2 (castration) x 5 (time of aging) factorial design using repeated measures on time was used on the pH values, thiol groups, and metmyoglobin. The data were analyzed by the PROC MIXED procedure of the SAS.

III. RESULTS AND DISCUSSION

The pH values, thiol groups, and metmyoglobin percentage obtained in steaks from bulls and steers are presented in Table 1.

Table 1 Values of pH, thiol groups, and metmyoglobin for the *Longissimus dorsi* muscle from bull and steer

Variable	Bull	Steer	P value
pH value	5.4 (0.03)[a]	5.3 (0.03)[b]	0.04
Thiol groups[§]	85.2 (3.27)	78.8 (3.27)	0.20
Metmyoglobin[£]	24.8 (0.70)	24.3 (0.73)	0.77

Legend: § = values expressed in nmol/mg of protein; £ = values expressed in %; [a,b]Different letters in the same row differ statically ($P < 0.05$).

An effect of castration was detected ($P < 0.05$) for the pH values. The steaks from bulls had higher ($P < 0.05$) pH values when compared to the steaks from steers. The bull may have a higher susceptibility to pre-slaughter stress, what could have affected glycogen deposition in muscle, leading to a more elevated ultimate pH [12]. High ultimate pH found in bull has been related to a higher frequency of dark-cutting in carcasses [13,14]. Similar result was found in another study done in our laboratory [15].

On the other hand, the castration did not affect ($P > 0.05$) the thiol groups and metmyoglobin, which are indicators of protein oxidation. This must be related to the similarity found for the fat thickness in the carcasses and for the antioxidant enzymes activities in meat from bulls and steers (data not shown).

There was an effect of the time of aging ($P < 0.05$) on the thiol groups and metmyoglobin (Fig. 1).

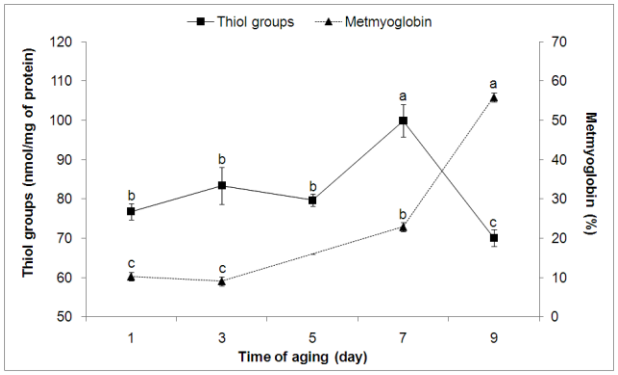

Figure 1. Thiol groups and metmyoglobin in *Longissimus dorsi* muscle of aged beef
Legend: [a,b,c]Different letters across the times of aging in the same variable differ statically ($P < 0.05$).

The thiol groups were similar ($P > 0.05$) until the 5th day, increased at the 7th day, and reached the minimum point at the 9th day. This suggests that no protein oxidation was observed in steaks from *LD* muscle under aerobiosis until the 5th day of aging. The proteolysis could explain the increase of thiol groups at the 7th day. A progressive increase of thiol groups in fish across the 47 hours has been attributed to protein degradation [16]. We believe that the proteolysis may have exposed the thiol groups at the 7th day, which were oxidized at the 9th day.

In turn, the metmyoglobin percentage remained constant until the 3rd day, increased at the 7th day, and reached the maximum point of 55.9% at the 9th day. This result is in agreement with those found in literature. A report showed that the metmyoglobin percentage in meat surface ranges from 25 to 50% after the 8th day of aging [17].

A decrease of thiol groups and an increase of metmyoglobin at the 9th day of aging point to a relationship between these two variables, as have been verified previously [5]. It is probable that the high permeability of the PVC film to the oxygen collaborated for this relationship [18,19], since both the formation of metmyoglobin and the oxidation of thiol groups are accelerated by the oxygen.

IV. CONCLUSION

The castration does not affect thiol groups and metmyoglobin in *LD* muscle from Nellore cattle. Also, the aging of the meat under aerobiosis condition accelerates the protein oxidation (thiol groups) and the conversion from myoglobin to metmyoglobin.

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