EFFECT OF FARM MANAGEMENT AND MIXING ANIMALS AS STRESSORS ON THE MUSCLE BIOMARKERS OF AUTOPHAGY AND OXIDATIVE STRESS IN BEEF

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I. INTRODUCTION

Animal management practices may influence the animal's stress at slaughter, with detrimental effects on the ultimate meat quality. Previous research performed in pigs has shown that the rearing management (indoor *vs* outdoor) and the mixing of unfamiliar animals prior to slaughter influence the mechanisms involved in the postmortem conversion of muscle into meat [1,2]. The objective of this work was to study the effect of different animal management treatments on farms (intensive *vs* semi-extensive) and during the transport and lairage prior to slaughter (mixing with unfamiliar animals *vs* unmixing) on the *postmortem* evolution of biomarkers of muscle metabolism in beef, with a special focus on variations in biomarkers of autophagy and oxidative stress, that may affect the process of meat quality acquisition.

II. MATERIALS AND METHODS

Twenty four yearling bulls of the "Asturiana de los Valles" breed (14-16 months old) were reared under two different finishing systems: intensive (indoor fattening with concentrate meal and barley straw *ad libitum)* or semi-extensive (outdoor management with pasture + 3.5 kg concentrate/day). Within each farm treatment, half of the animals were mixed with unfamiliar individuals during transport and lairage prior to slaughter (mixed) and half of the animals were kept together (unmixed). Muscle samples were taken from the *Longissimus dorsi* of each animal at 2h, 8h and 24h *postmortem*. ATP level was assessed using the ATP Bioluminescent Assay Kit (FLAA, Sigma-Aldrich). Biomarkers of oxidative stress [Total antioxidant activity (TAA), Lipid peroxidation (LPO)] and autophagy (Beclin-1, LC3-I, LC3-II) were analysed following the procedures described by Potes et al. [3]. The effect of farm management (F), mixing treatment (M), postmortem time (t) and their interactions were analysed by ANOVA using the SPSS. When significant, differences were analysed with the Tukey post-hoc test (Friedman test for non-normal data).

III. RESULTS AND DISCUSSION

Autophagy biomarkers (Beclin-1, ratio LC3-II/LC3-I) were active under all the studied conditions (Fig. 1). There were not significant changes due to the animal management at the farm (F) or due to the mixing treatment (M) but there was a significant effect of *postmortem* time (t) on the activity of Beclin-1, which showed a decreasing rate along the studied postmortem period (2h-8h-24h). This decreasing rate was significantly higher in the muscle of animals reared under semi-extensive conditions (there was a significant interaction F x t, $P< 0.001$) due to a higher initial level and a faster exhaustion of autophagy in the muscle cells of bulls reared outdoors. This could reflect a different muscle fiber development due to the rearing conditions and also a different reaction to the oxidative stress produced by the cut of oxygen at slaughter.

The ratio of conversion LC3-II/LC3-I showed a decreasing pattern during the first 24h *postmortem*, being a significant change ($P< 0.05$) from the unmixing treatments (Fig. 1). TAA was higher in the group of animals from the "intensive-mixing" group (Fig. 2), in which there was a high variability of results because some animals exhibited extreme results of oxidative stress (high TAA and also high LPO). Furthermore, in the "intensive-mixing" group the ATP availability in the muscle was significantly lower ($P< 0.001$) than in any other treatment (Fig. 2), which could be the results of a higher stress reaction of these animals at slaughter.

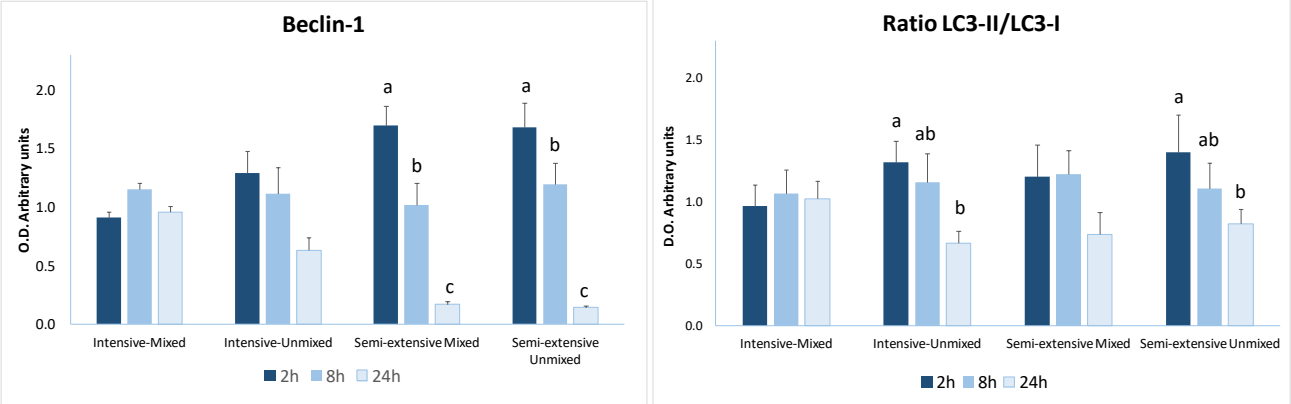

Figure 1. *Postmortem* evolution of biomarkers of autophagy (Beclin-1 and LC3-II/LC3-I ratio) in the muscle cell.

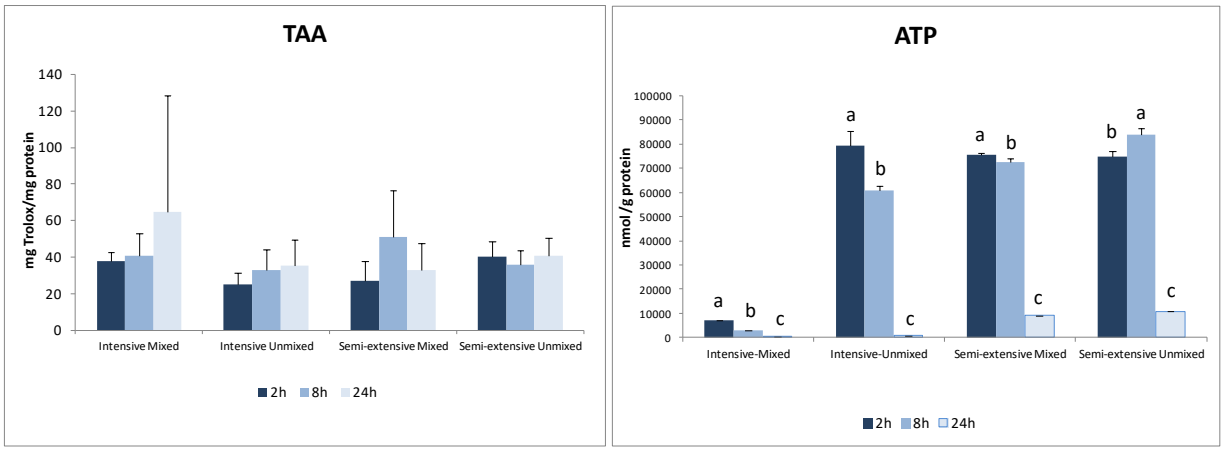

Figure 2. *Postmortem* evolution of TAA and ATP level in the muscle cell.

IV. CONCLUSION

Autophagy activity takes place in the muscle cell during the first 24 h *postmortem* as a mechanism against the increased oxidative stress derived from the deprivation of oxygen and nutrients due to the animal's exsanguination at slaughter. Furthermore, the total antioxidant activity of the tissue increases as a reaction to the oxidative stress. These reactions are affected by the animal management at farm (intensive or semi-extensive) and by the procedure of mixing unfamiliar animals prior to slaughter.

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