Effect of intermediate ultimate pH beef over aging time on *Longissimus lumborum* muscle proteome from grass-fed Nellore

Monique M. Krauskopf[1], Daniel S. Antonelo[2], Chimenes D. L. Araújo[1], Eduardo F. Delgado[3], Carmen J. Contreras-Castillo[1*]

[1] Department of Food Science and Technology, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, 13418-900, Brazil

[2] Lipid Marker Omics Sciences, Pirassununga, SP, 13638-407, Brazil

[3] Department of Animal Science, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP 13418-900, Brazil

*Corresponding author email: ccastill@usp.br

I. INTRODUCTION

The ultimate pH (pHu) plays an important role in beef quality. Several factors such as animal diet, exercise and pre-slaughter stress can result in muscle glycogen depletion and cause an abnormal pHu beef (> 5.80) [1]. In Brazil, the incidence of beef classified as intermediate (5.8 to 6.19) or high pHu (≥ 6.2) represents approximately 40% of production, resulting in large economic losses [2]. Therefore, the aim of this work was to evaluate the effect of intermediate pHu beef over aging time on *Longissimus lumborum* (LL) muscle proteome from grass-fed Nellore (*Bos indicus*) bulls.

II. MATERIALS AND METHODS

Three LL muscles from grass-fed Nellore bulls (30 - 35 months old) classified as intermediate pHu (pHu 5.8 to 6.19) were obtained from a commercial slaughterhouse. The muscles were divided into 2.5 cm thick steaks and assigned to a treatment: 1-d (72 h *post-mortem*) and 14-d of vacuum aging at 4°C. Samples from each aging time were stored at −80 °C for proteomic analysis. Protein extraction was performed as described by Wiśniewski *et al*. [3]. LC-MS/MS analysis was performed on a Xevo G2-QTof mass spectrometer (Waters Coorporation) coupled directly to the chromatographic system. Differentially abundant proteins (DAPs) over aging time were defined through volcano plot analysis (fold change ≥ 1.2; $P \leq 0.05$). Enriched Gene Ontology terms and pathways were investigated using the open-source tool Metascape® ($P \leq 0.05$, minimum overlap of 3, and enrichment factor > 1.5).

III. RESULTS AND DISCUSSION

PCA discriminated beef at 1-d and 14-d aging (Fig. 1A). Volcano plot analysis (Figure 1B) revealed 26 DAPs between aging times comparison, of which 12 were over abundant at 1-d aging and 14 were over abundant at 14-d aging. Enrichment analysis revealed 10 enriched terms (Figure 1C), in which most of them were related to energy metabolism and muscle structure. Succinate-CoA ligase subunit alpha (SUCLG1), NADH dehydrogenase ubiquinone flavoprotein 2 (NDUFV2), ubiquinone biosynthesis monooxygenase COQ6 (COQ6) and NADH dehydrogenase ubiquinone 1 subunit C2 (NDUFC2) were abundant at 1-d aging indicating increased oxidative metabolism, as also observed by Zhai et al. [4]. Suggesting increased oxidative stress in meat with intermediate pH resulting in reduced proteolysis and degradation of structural proteins. The overabundance of candidate biomarkers for tenderness such as aldehyde dehydrogenase (ALDH2), myozenin-1 (MYOZ1), malate dehydrogenase, mitochondrial (MDH2), troponin T (TNNT1) and underabundance of heat shock protein HSPA5 observed in intermediate pHu beef at 14-d aging compared to 1-d aging are indicative of a delay in tenderization, which partially explain the toughness of intermediate pHu beef, as reported by [1, 5].

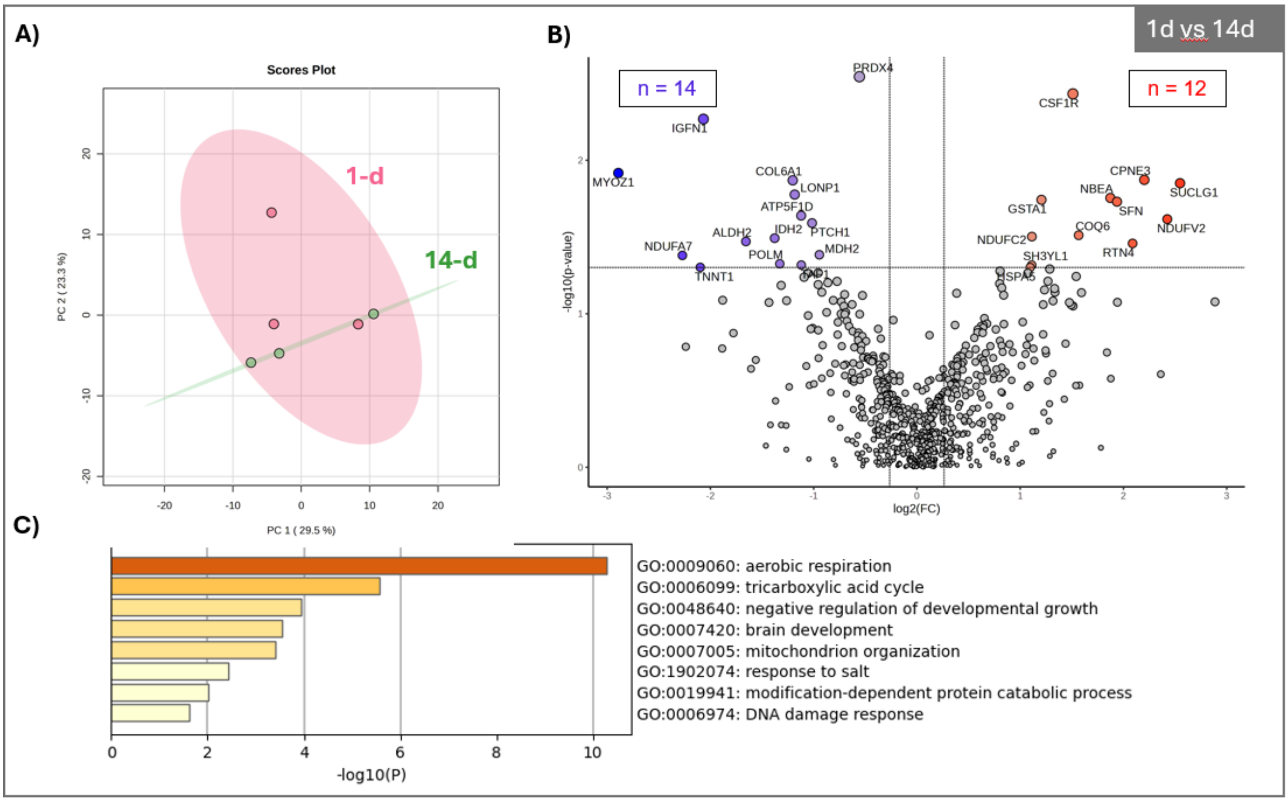

Figure 1. A) Principal Component Analysis (PCA); B) Volcano plot showing DAPs between 1 and 14 days of aging; C) Bar chart of significantly enriched GO cluster terms according to *P*-values ($P \leq 0.05$).

IV. CONCLUSION

The main proteomic changes of intermediate pHu beef over aging time are related to energy metabolism and muscle structure, revealing some proteins important for the main meat quality attributes, such as beef color and tenderness.

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