Omics strategies to differentiate *Longissimus lumborum* beef within three ultimate pH groups

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I. INTRODUCTION

The influence of ultimate pH (pHu) ranges on quality parameters of aged *Longissimus lumborum* steaks from Nellore bulls has been studied (Barón et al., 2021; Lomiwes et al., 2013; Ramanathan et al., 2020). However, there is a gap of omics studies applied for intermediate pHu (5.8-6.2) muscles aged for long periods. Our objective was to assess the changes on lipidome and metabolome profiling of *Longissimus lumborum* beef of Nellore bull muscle with different pHu (normal [< 5.8], intermediate [5.8 to 6.2], and high [\geq 6.2]) and postmortem aging (3 days and 21 days). This study especially fills a gap of knowledge on how would the aged intermediate pHu muscle, which is tougher, be differentiated in terms of lipids and metabolites that are likely to influence their sensory attributes.

II. MATERIALS AND METHODS

Pasture-finished Nellore bulls (n = 162) with 30-35 months of age, 4 to 6 permanent incisor teeth, were tracked since humanitarian slaughter until carcass processing in a commercial slaughterhouse. Nine *Longissimus lumborum* muscles were selected and classified based on the pHu after 72 h postmortem within pHu groups: 3 as Normal (< 5.8), 3 as Intermediate (5.8 to 6.2), and 3 as High (\geq 6.2) (Lomiwes et al., 2013). Two 2 cm steaks were cut from each muscle and were randomly assigned to two aging periods: 3 days [3-d] and 21 days [21-d]. They were individually vacuum-packed, totalizing 18 steaks (3 animals per each 3 pHu groups at each 2 aging periods). Postmortem aging was performed at 2 °C, and then the muscles were stored at -80 °C until analysis. Lipids were extracted for targeted lipid profiling, which was performed using discovery MRM-profiling methods and instrumentation (Xie et al., 2021). Mass spectrometry data were acquired (Antonelo et al., 2022), metabolites were acquired at 300 K using a Bruker Avance 14.1 T spectrometer (Bruker Corporation) at 600.13 MHz. Lipidome and metabolome data were uploaded to MetaboAnalyst 5.0, and data were Pareto-scaled. Hierarchical clustering heatmaps were performed. Pairwise comparisons were conducted: 1) High versus Normal, 2) High versus Intermediate, and 3) Intermediate versus Normal.

III. RESULTS AND DISCUSSION

The samples within the pHu groups tended to be clustered together at both postmortem aging periods, as exemplified for metabolites in the dendrograms shown in Figure 1. As major results, carnitines acylated with C12:0 and C14:0 fatty acids were identified as potential biomarkers of the intermediate pHu-muscle regardless the aging period. The normal pHu-muscle showed higher concentrations of metabolites of the glycogenolysis and glycolysis pathways, including glucose, mannose, and pyruvate. The high pHu-muscle was differentiated by the higher concentrations of fumarate (a metabolite of the

tricarboxylic acid cycle), formate (a metabolite of amino acid degradation), and acetate (produced by the action of reactive oxygen species on pyruvate). Interestingly, the concentration of arginine at early postmortem aging (3-d) may influence the previously reported improved tenderness in normal and high pHu-muscles at later aging (21-d).



Figure 1. Hierarchical clustering heatmaps of the metabolome profiling of *Longissimus lumborum* muscles within ultimate pH groups (normal, intermediate, and high) at 3 days (A) and 21 days (B) of aging.

IV. CONCLUSION

Finding markers associated with biochemical changes that influence sensory attributes in meat is important for: a) using their levels to monitor aspects of meat production chain, such as antemortem stress level; and b) studying strategies to modulate the mechanisms that affect such markers levels to avoid meat defects. In our study, knowledge of postmortem biochemical changes of long-term aged beef within different pHu groups was raised based on preliminary data, which is essential to understand the mechanisms underpinning bull meat defects. Further studies should be encouraged to confirm our findings.

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