SERIAL TIME ANALYSIS OF *POST-MORTEM* MUSCLE UNVEILS A DISPARITY IN THE BIOMARKERS AND BIOCHEMICAL PATHWAYS UNDERPINNING DARK-CUTTING BEEF DEVELOPMENT

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I. INTRODUCTION

The use of proteomics to discover biomarkers to accurately assess meat quality and its defects has opened new avenues for meat quality management [1]. Proteomics was successfully applied to enlighten our knowledge on the underlying mechanisms of muscle into meat conversion, including the occurrence of high ultimate pH (pHu) meat, also known as Dark Firm and Dry or dark-cutting meat [2]. However, the available proteomics studies describing DFD meat-related proteome were mainly focused on muscle samples taken after 24-hour post-slaughter. Those studies delivered some insights about this quality defect, but there is a need to better understand the mechanisms by assessing the dynamic changes in the same muscle over time, considering the early *post-mortem* periods. This study is designed in that context and aims for the first time to use a serial time analysis to evaluate the dynamic changes between high and normal muscle pHu at 30 min, 9 h and 44 h *post-mortem*.

II. MATERIALS AND METHODS

Ten carcasses classified as normal (pHu < 5.8; n = 5) or high (pHu ≥ 6.2; n = 5) pHu beef from pasture-finished Nellore (*Bos indicus*) bulls, ranging from 30 to 35 months of age (4 to 6 permanent incisors teeth) and 349 ± 31 kg of hot carcass weight, were obtained from a commercial meat processor. *Longissimus thoracis* (LT) muscle samples (~ 30 g) were taken from the carcasses (between the 10th and 11th ribs) at 30 min, 9 h and 44 h *post-mortem*, immediately snap frozen in liquid nitrogen and stored at -80 °C for further proteomics analysis. A shotgun proteomics approach was carried out as described by Lamri *et al.* [3]. The quantitative abundances of the proteins were statistically analysed to identify the differentially abundant proteins (DAPs) at a level- of 5% and fold change ≥ 1.2. The DAPs between high and normal-pHu beef across *post-mortem* times were analysed by the webservice Metascape® (https://metascape.org/) bioinformatics tool (P ≤ 0.05, minimum overlap of 3, and enrichment factor > 1.5) to identify the enriched pathways and their comparison by means of hierarchical heatmap clustering.

III. RESULTS AND DISCUSSION

The shotgun proteomics approach allowed quantifying 863 unique proteins with high confidence and accuracy (> 2 peptides at an FDR of 1%), in which 33, 181 and 37 were differentially abundant between high and normal-pHu beef at 30 min, 9 h and 44 h *post-mortem*, respectively. The hierarchical heatmap clustering and comparison of the total DAPs revealed “generation of precursor metabolite and energy” and “peptide metabolic process” as major commonly enriched Gene Ontology (GO) terms whatever the *post-mortem* time (Fig. 1). Interestingly, “tricarboxylic acid cycle” (TCA) term was enriched in normal-pHu beef at 30 min and in high pHu at 9 h *post-mortem*. Moreover, “regulation of actin filament-based

process", "monocarboxylic acid metabolic process", "small molecule catabolic process", "protein catabolic process", "regulation of protein stability", "muscle cell development" and "proton transmembrane transport" were significantly and exclusively specific to the LT muscle proteome at 9 h *post-mortem*. Furthermore, "regulation of oxidative stress-induced intrinsic apoptotic signalling pathway" was exclusively enriched in normal-pHu beef at 44 h *post-mortem*. The findings revealed overall major dynamic changes in the *post-mortem* muscle proteome related to dark-cutting beef and disparity in the changing pathways over time. The results evidenced for the first-time important changes at 9h *post-mortem* compared to the very early (30 min) and late '48h) sampling times.

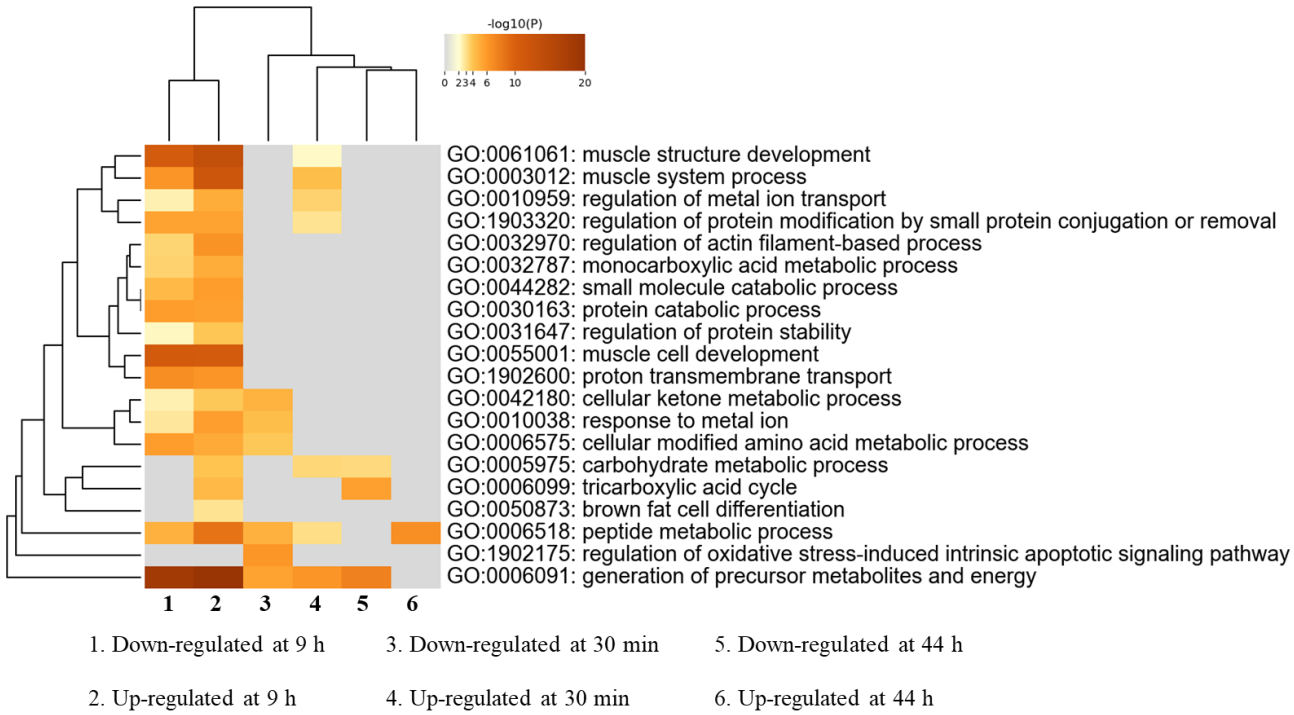

Figure 1. Hierarchical heatmap clustering to compare the enriched GO terms between the differentially abundant proteins from high and normal-pHu beef across *post-mortem* time. 1, 3 and 5) up-regulated in normal-pHu beef at 9 h, 44 h and 30 min, respectively; 2, 4 and 6) up-regulated in high beef at 9 h, 44 h and 30 min, respectively.

IV. CONCLUSION

A strong interconnectedness between the proteins and the functional pathways to which they belong was depicted in early *post-mortem* muscle of both normal and high pHu muscle samples. Insights of how these complex and interconnected pathways are changing over time revealed for the first time a disparity in the molecular signature at interplay. Overall, generation of precursor metabolites and energy pathways seemed of pivotal role in driving high pHu development during *post-mortem*.

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