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EARLY *POST-MORTEM* DISCRIMINATION BETWEEN FEEDLOT-FINISHED NELLORE BULLS WITH NORMAL AND HIGH ULTIMATE MUSCLE pH USING MUSCLE LIPID BIOMARKERS

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I. INTRODUCTION

Fresh purchasing decisions are primarily driven by lean color, with bright cherry-red appearance generally perceived by consumers as a positive indicator of meat freshness and wholesomeness [1]. Higher-than-normal ultimate pH (pHu) resulting from a stress-related decrease of muscle glycogen stores and attenuated *post-mortem* metabolism have been associated with dark-cut beef. As a result, carcasses with darker lean are generally downgraded during the process of grading, leading to significant economic losses globally for the beef sector [2]. Thus, it is essential to avoid failures in the categorization of carcasses according to the pHu and develop early *post-mortem* tools for their monitoring. If available, this would provide the industry with the ability to minimize the occurrence of this quality aberration, or at the very least, help us better understand how beef quality development is controlled *post-mortem*. In this sense, some highly accurate predictive tools, such as lipidomics, can be used to better understand pHu development at a molecular level, proposing molecular biomarkers to improve their predictions. We hypothesized that early post-mortem muscle lipidome segregates between pHu classes. Therefore, the aim of this study was to create an early post-mortem muscle lipid-based biomarker panel to discriminate between normal and high muscle pHu from feedlot-finished Nellore bulls.

II. MATERIALS AND METHODS

Twenty-eight carcasses classified as normal (pHu < 5.8; n = 14) or high (pHu ≥ 6.0; n = 14) pHu beef from feedlot-finished Nellore (*Bos Indicus*) bulls, ranging from 18 to 24 months of age, were obtained at a commercial slaughterhouse. Samples (~ 30 g) were excised from the *Longissimus thoracis* (LT) muscle (11th rib level) at 30 min and 44 h *post-mortem* and were immediately frozen in liquid nitrogen and stored at -80 ºC for further lipidome analysis and pH measurement, respectively. Muscle pHu was determined using the iodoacetate method as described by Bendall [3]. Each LT muscle sample (n = 20 per pHu class) collected at 30 min *post-mortem* were ground in liquid nitrogen for lipid extraction using a method reported by Bligh and Dyer [4]. Targeted lipid profiling was performed using Multiple reaction monitoring (MRM)-profiling methods and instrumentation as reviewed by Xie et al. [5]. Further details about lipidome analysis have been described by Antonelo et al. [6]. Lipidome data were uploaded to Metaboanalyst 6.0 (https://www.metaboanalyst.ca/) and data were Pareto-scaled prior to statistical and bioinformatics analyses. Partial least squares discriminant analysis (PLS-DA) was performed for treatment discrimination. Moreover, receiver operating characteristic (ROC) curve analysis was performed to create a predictive model for evaluating the performance of putative biomarkers for LT muscle pHu.

III. RESULTS AND DISCUSSION

Out of the 3,437 MRMs scanned in a pooled sample for identifying the detectable lipids, 464 had intensities of at least 1.3-fold higher than the blank and were used for interrogating individual samples. The PLS-DA (Figure 1A) revealed distinct clusters between muscle lipidome from normal and high pHu muscle (Accuracy = 0.96; R^2 = 0.86; Q^2 = 0.73). Based on the ROC curve (area under the curve = 0.962), a model was created to predict LT muscle pHu, which obtained a predictive accuracy from 80.8% to 88%, depending on the number of putative biomarkers used (Figure 1B). The model misclassified only 4 (2 high and 2 normal pHu) out of the 28 samples (Figure 1C), in which, based on the mean importance measure of the model (Figure 1D), were mainly related to the acylcarnitines (CAR; upregulated in high pHu) and phosphatidylinositols (PI; upregulated in normal pHu). Antonelo et al. [6] also reported overabundance of CAR in high pHu at 30 min post-mortem, which suggests that during early postmortem metabolism, muscle with a high pHu may try to compensate for low glycogen concentrations by oxidizing fatty acids to generate ATP for maintaining energy homeostasis in the tissues.

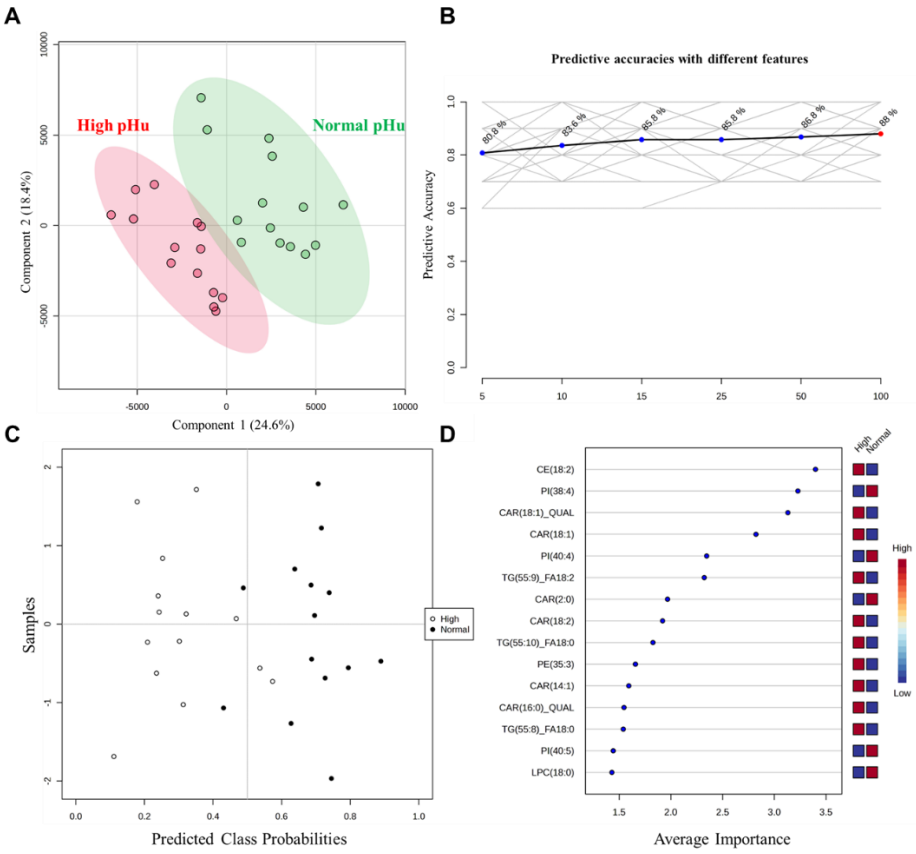


Figure 1. Muscle lipidome analysis from feedlot-finished Nellore bulls with normal (pHu < 5.8) and high (pHu ≥ 6.0) ultimate muscle pH (pHu). A) Partial least squares discriminant analysis (PLS-DA); B) Model predictive accuracy; C) Predicted class probabilities; D) Top 15 important features to the model.

IV. CONCLUSION

The early *post-mortem* muscle lipidome from normal and high muscle pHu has a discriminating profile, which allowed the creation of a preliminary predictive model to discriminate them right after animal slaughter. Therefore, it can be used as a tool to assist and/or replace pHu classification techniques performed in the late *post-mortem*. Moreover, it may allow the implementation of techniques to promote effective decline of muscle pH during the early *post-mortem* period to minimize the occurrence of pH defects.

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