EARLY *POST-MORTEM* DISCRIMINATION BETWEEN BEEF TENDERNESS CLASSES IN FEEDLOT NELLORE BULLS USING MUSCLE LIPID BIOMARKERS

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I. INTRODUCTION

Tenderness is one of the most important traits related to meat quality. While consumers are able to discriminate among tenderness variations and are willing to pay a premium for tender beef [1], there is a significant inconsistency in this trait. Thus, omics approaches have been studied to better understand the causes of variability in tenderness and the biochemical mechanisms underlying tenderization. In a previous study, Antonelo et al. [2] observed muscle lipidome, mainly lipids from phosphatidylethanolamine class that are implicated in cellular apoptosis via mitochondrial permeability transition initiated by reactive oxygen species, affects the tenderness development. Therefore, in a dedicated study, we hypothesized that early *post-mortem* muscle lipidome segregates between tenderness classes. The aim of this study was to create an early *post-mortem* muscle lipid-based biomarker panel to discriminate between tender and tough beef.

II. MATERIALS AND METHODS

Carcasses from feedlot Nellore (*Bos indicus*) bulls (n = 100; from 20 to 24 mo age) were obtained at a commercial slaughterhouse. *Longissimus thoracis* (LT) muscle (11^{th} rib level) samples were collected at 30 min *post-mortem* for further lipidome analysis. After 48 h of chilling (0°C to 2°C), the left side of each carcass was ribbed between the 12^{th} and 13^{th} ribs, and a 2.5-cm-thick LT sample of each carcass was obtained for Warner-Bratzler shear force (WBSF) analysis [3]. The WBSF values from all samples were utilized to identify the carcasses with the lowest (most tender; < 60 N) and highest (toughest; > 90 N) WBSF to create 2 beef tenderness classes (n = 10 per class; tender [average = 51.4 N; minimum = 39.7 N; maximum = 58.4 N; SD = 5.38] and tough [average = 100.9 N; minimum = 92.2 N; maximum = 116.5 N; SD = 8.1]). Each LT muscle sample (n = 10 per tenderness 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. [2]. Lipidome data were uploaded to Metaboanalyst 6.0 (https://www.metaboanalyst.ca/) and data were auto scaled prior to statistical and bioinformatics analyses. Principal component analysis (PCA) and hierarchical clustering heatmap were performed. Moreover, receiver operating characteristic (ROC) curve analysis was performed to create a predictive model for evaluating the performance of putative biomarkers for beef tenderness.

III. RESULTS AND DISCUSSION

Out of the 1366 MRMs scanned in a pooled sample for identifying the detectable lipids, 300 had intensities of at least 1.3-fold higher than the blank and were used for interrogating individual samples. The PCA (Figure 1A) and hierarchical clustering heatmap (Figure 1B) analyses revealed distinct

clusters between muscle lipidome from tender and tough beef. Based on the ROC curve (Figure 1C), a model was created to predict the beef tenderness, which obtained a maximum predictive accuracy of 73.3% using 50 putative biomarkers (Figure 1D), misclassifying only 4 out of the 20 samples.

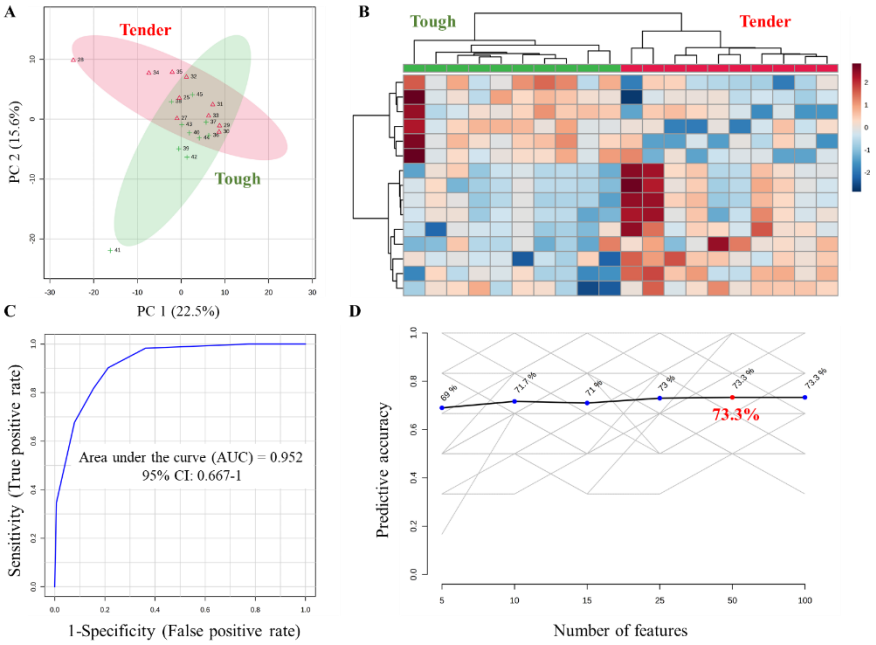

Figure 1. Muscle lipidome analysis from tender and tough beef. A) Principal component (PC) analysis; B) Top 15 hierarchical clustering heatmap analysis; C) ROC curve; D) Predictive accuracy of the model.

IV. CONCLUSION

Early *post-mortem* muscle lipidome from tender and tough beef has a discriminating profile, which allowed creating a preliminary predictive model to discriminate them right after animal's slaughter. Therefore, this early *post-mortem* muscle lipid-based biomarker panel can be used as a tool to assist and/or replace tenderness classification techniques performed in the late *post-mortem,* increasing the feasibility and reliability of the beef tenderness certification.

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