BEEF ULTIMATE PH-DEPENDENT BIOMARKERS IDENTIFIED BY TWO-DIMENSIONAL GEL-BASED PROTEOMICS

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I. INTRODUCTION

The ultimate pH (pH_u) is a relevant indicator of beef quality. At the slaughter, bulls regularly present a reduced concentration of muscle glycogen in response to stressors, which alters *post mortem* ATP and lactate levels, hindering the pH decline, leading to an abnormal beef pH (> 5.80). The pH decline *post mortem* has a remarkable impact on muscle biochemistry and structure, influencing beef tenderness and color [1, 2]. In the last years, many studies have used proteomics approaches to identify differentially abundant proteins (DAPs) associated with beef quality with different pH_u values, but using samples with 1 to 24 h *post mortem* [3]. This study aimed to identify potential biomarkers on proteome of *Longissimus thoracis* (LT) muscle obtained from Nellore bulls at 30 min *post mortem* with different pH_u and slaughter seasons (summer and winter).

II. MATERIALS AND METHODS

Eighteen carcasses classified as Normal (≤ 5.79, n = 6), Intermediate (5.80 to 6.19, n = 6) and High-pH_u (≥ 6.20, n = 6) from pasture-finished Nellore (*Bos Indicus*) bulls, ranging from 30 to 35 months of age (4 to 6 permanent incisors teeth) were obtained in Brazilian summer (1st slaughter) and winter (2nd slaughter) seasons from a commercial abattoir. LT muscle samples (~ 15 g) were obtained from the carcasses (10th ribs) at 30 min *post mortem*, frozen in liquid nitrogen and stored at -80 °C until the proteomic analysis. Protein extraction was carried out using 50 mg of LT muscle sample in 500 µL of lysis buffer. Bradford method was performed using BSA as standard. The 2-DE maps were obtained using IPG strips of 13 cm pH 4-7, loaded with 400 ug of protein and 12,5% SDS-PAGE. Gels were stained with Coomassie Brilliant Blue G-250, and analyzed using ImageMaster 2D Platinum 7.0 software. Spots differentially expressed ($P < 0.05$, 1.5-fold intensity) were excised from the gels and digested with trypsin. The protein was identified by mass spectrometry (n-ESI-QTOF MS/MS) and the relative quantification of each protein was determined through the emPAI and log FC ratios at either time point were defined as up- or downregulated proteins, respectively. The STRING database, via the Cytoscape were used to the potential protein-protein interactions (PPI) between the DAPs.

III. RESULTS AND DISCUSSION

In the 2-DE proteomic profiles, more than 300 protein spots were detected (Fig. 1a). We identified DAPs (25 for 1st and 12 for 2nd slaughter, respectively), up- or downregulated. Eight DAPs were commonly identified in both slaughters (Fig. 1b). PGM1, ENO3, PKM, GAPDH, TNNT1 and ACTA1 were enrichment in Intermediate- and High-pH_u in comparison to Normal-pH_u samples, whereas LDHA was downregulated in Intermediate-pHu *vs.* Normal-pH_u and ATP5B was downregulated in High-pH_u *vs.* Intermediate-pH_u for both slaughters, suggesting an accentuated rate of *post mortem* glycolysis. Gene ontology (GO) results revealed enrichment for processes that are involved

predominantly in small molecule metabolic process (GO:0044281), carbohydrate metabolic process (GO:0005975) and phosphorylation (GO:0016310) (Fig. 1c).

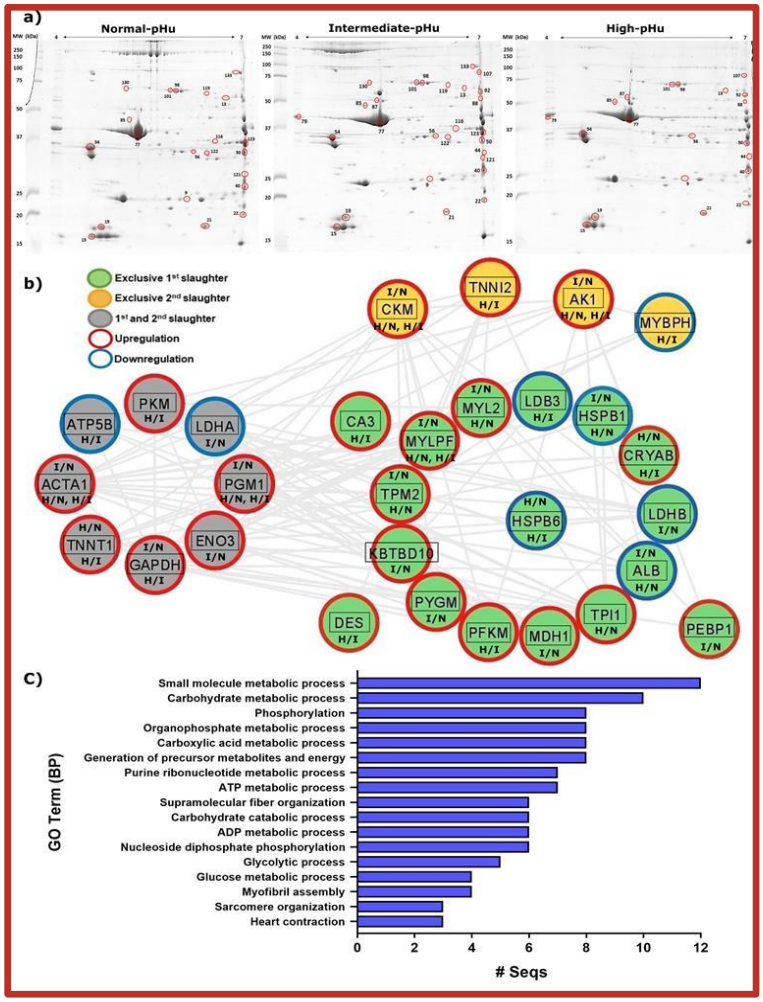

Figure 1. Representative 2-DE gels for both slaughters (a), interaction protein network (b) and GO biological process of DAPs (c). Ratios: I/N, H/N and H/I, N: Normal-pH_u, I: Intermediate-pH_u and H: High-pH_u. Protein name: ACTA 1 - Actin alpha 1; AK1 - adenylate kinase isoenzyme 1; ALB - Albumin; ATP5B - ATP synthase subunit beta; CA3 - Carbonic anhydrase; CKM - Creatine kinase; CRYAB - Alpha-crystallin B chain; DES - Desmin; ENO3 - Phosphopyruvate hydratase; GAPDH - Glyceraldehyde-3-phosphate dehydrogenase; HSPB1 - Heat shock protein beta-1; HSPB6 - Heat shock protein; KBTBD10 - Kelch like family member 41; LDB3 - LIM domain binding 3; LDHA - L-lactate dehydrogenase; LDHB - L-lactate dehydrogenase; MDH1 - Malate dehydrogenase; MYBPH - Myosin binding protein; MYL2 - Myosin light chain 2; MYLPF - Myosin regulatory light chain 2; PEBP1 - Phosphatidylethanolamine; PGM1 - Phosphoglucomutase-1; PFKM - ATP-dependent-6-phosphofrutokinase; PKM - Pyruvate kinase; PYGM - Alpha-1,4 glucan phosphorylase; TPI1 - triosephosphate isomerase; TPM2 - Tropomyosin 2; TNNT1 - Troponin-T; TNNI2 - Troponin I2.

IV. CONCLUSION

Intermediate- and High-pH_u promote changes in the protein profiles involved in biological process including metabolic proteins and glycolytic enzymes, which corroborate their contribution to early post mortem metabolic changes and as potential biomarkers to predicting beef quality.

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