PROTEOME AND METABOLOME OF EARLY POSTMORTEM *LONGISSIMUS DORSI* TO EXPLORE ULTIMATE PH AND PORK QUALITY

E. Zuber[1*], A. Outhouse[1], E. Huff-Lonergan[1], E. Steadham[1], and S. Lonergan[1],

[1]*Animal Science, Iowa State University, Ames, IA, USA*,

*bzuber@iastate.edu

I. OBJECTIVES

Extent of pH decline relies on postmortem (PM) muscle metabolism. Glycolytic mechanisms maintain metabolism and may influence pork quality. The objective was to identify metabolomic and proteomic features that are associated with variation in ultimate pH pork loin. The hypothesis was that a combination of elements in early PM *Longissimus dorsi* (LD) proteome and metabolome can be used to understand variation in pH decline.

II. MATERIALS AND METHODS

Pigs ($N = 47$) were harvested using standard industry procedures. Samples were removed from the LD at 45 min PM, snap-frozen in liquid nitrogen, powdered, and stored at −80°C. A portion of the LD was used for pH and quality measurements (lightness, purge loss, star probe, and intact desmin) after 14 d of aging. Densitometry analysis of immunoblots was used to quantify intact desmin, resolved in the whole muscle extracts from day-14 samples. Groups were classified as normal pH (NpH) ($\mu = 5.59$, 5.53–5.67; $n = 10$) and low (LpH) ($\mu = 5.42$, 5.38–5.45; $n = 10$) at 14 d PM for metabolomic and proteomic analyses. Nontargeted, polar metabolites from 45 min PM were identified using gas chromatography-mass spectrometry. Proteins were solubilized in sarcoplasmic extraction buffer from 45 min PM samples. Two-dimensional difference in gel electrophoreses experiments were conducted in duplicate. Differentially abundant spots were identified with MALDI-MS. Statistical analysis for pH and quality was done with GLM procedures of SAS version 9.4 (SAS Institute Inc., Cary, NC) and fixed effect of 14 d pH classification. Statistical differences between NpH and LpH were reported as significant ($P < 0.05$). Metabolomics and proteomics were analyzed through MetaboAnalyst (Xia Lab, McGill, CA) and Melanie 9 (Cytiva, Marlborough, MA), respectively, using a Student *t* test. Differential abundance between NpH and LpH were reported as significantly different fold changes (FC = NpH/LpH; $P < 0.1$).

Table 1.
Spots denoted as fold change (NpH/LpH; $P < 0.1$).

ID	FC	*P* value
Fructose Bisphosphate Aldolase (ALDO) (Spot 152[1], 156[1], 155[1], 151[1])	1.25, 1.29, 1.30, 1.27	0.04, 0.07, 0.07, 0.09
Pyruvate Kinase (PK) (Spot 124[1], 121[1], 63[2])	1.15, 1.25, 1.52	0.10, 0.05, 0.08
α 2-Heat Shock Glycoprotein (HSG) (Spot 128[1])	1.33	0.06
Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) (Spot 57[1])	1.49	0.02
L-Lactate Dehydrogenase (LLDH) (Spot 49[1])	1.56	0.03
Heat Shock Protein 70 (HSP70) (Spot 79[1])	1.44	0.13
β-enolase (Spot 39[2])	1.50	0.08
α-Actin (Spot 86[1], 41[2], 40[2], 42[2])	1.93, 2.07, 2.32, 1.76	0.01, 0.04, 0.04, 0.06

- IPG strip pH 3–10.
- IPG strip pH 4–7.

III. RESULTS

Ultimate pH classification did not affect 45 min PM pH ($P = 0.64$); 14 d pH was different between groups ($P < 0.01$). NpH LD were darker (L: NpH, 47.63; LpH, 50.58; $P = 0.03$) and had less purge loss (NpH, 2.40; LpH, 3.84; $P < 0.01$), lower star probe (NpH, 5.36; LpH, 6.31; $P < 0.01$) and less intact desmin (NpH, 1.43; LpH, 2.36; $P = 0.06$). Fructose 6-phosphate (FC = 0.76, $P = 0.06$) and lactate (FC = 0.59, $P = 0.09$) were greater in LpH; glycerate 3-phosphate (FC = 1.39, $P = 0.11$), pyruvate (FC = 1.98, $P = 0.01$), and malate (FC = 1.59, $P < 0.01$) were greater in NpH. Proteins involved in contraction, metabolism, and heat stress response were greater in NpH (Table 1).

IV. CONCLUSION

Subtle changes in ultimate pH can influence quality even when early PM pH is not different. However, differences in the proteome and metabolome at this early time PM are associated with differences in the extent of pH decline, and therefore these features deserve evaluation.

Keywords: early postmortem, metabolome, pork quality, proteome