AN INTEGRATIVE OMICS APPROACH TO UNDERSTAND SARCOPLASMIC RETICULUM'S ROLE IN ELEVATED LEVELS OF FREE CALCIUM IN BROILER WOODY BREAST

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I. OBJECTIVES

Woody breast (WB) is a myopathy observed in broiler breast meat (*Pectoralis major*) that results in rubbery texture. A previous study from our lab noted elevated levels of free calcium (Ca^{2+}) and sarcomere shortening in WB meat compared to normal. We hypothesize that a sarcoplasmic reticulum (SR) dysfunctionality associated with WB may result in the additional leakage of intracellular Ca^{2+} into the sarcoplasm. The objective of this study was to utilize an integrative omics (lipidomics/proteomics) approach to investigate the functionality/integrity of WB SR.

II. MATERIALS AND METHODS

Fourteen Ross line broiler breast fillets (7 severe WB and 7 normal) were collected, packaged, and frozen at 8 h postmortem from a commercial processing plant. The SR fractions of the samples were extracted via ultracentrifugation through discontinuous sucrose gradients. The lipid was extracted from the SR fractions and utilized for comprehensive phospholipid profile analysis (lipidomics) through electrospray ionization tandem mass spectrometry. The protein was extracted from the SR fractions in urea, and protein concentration was determined. Proteins were separated using high-performance liquid chromatography and identified using an Orbitrap-based mass spectrometry system (proteomics). Level of significance for statistical analysis was set at $P < 0.05$ for both omics data.

III. RESULTS

Sixty-six phospholipid species and 677 proteins (false discovery rate = 0.05) showed differential abundance. Lipidomics data revealed that WB SR had less relative percentage of phosphatidylcholine and more phosphatidylethanolamine (PE), phosphatidylserine (PS), and lysophosphatidylcholine compared to normal SR ($P < 0.05$). Proteomics data revealed an upregulation of Ca^{2+} transport proteins (e.g., sarcoplasmic/endoplasmic reticulum calcium adenosine triphosphatase) and a downregulation of proteins responsible for Ca^{2+} release and signaling (e.g., ryanodine receptors; $P < 0.05$) in WB SR. There was no difference in protein abundance for Ca^{2+} storage proteins (e.g., calsequestrin). Interestingly, carboxylic ester hydrolase (representing acetylcholinesterase [AChE] activity) exhibited a 7.62-fold increase in WB SR ($P < 0.01$), and there was also an upregulation of phospholipase A2 in WB SR ($P < 0.01$).

IV. CONCLUSION

Changes in phospholipid composition and increases in phospholipid catabolism in SR may be partly responsible for the increased free Ca^{2+} levels in WB meat. WB SR membrane integrity may be compromised due to an upregulation of phospholipase A2, resulting in the hydrolysis of phosphatidylcholine, the main building block of lipid bilayers, into lysophosphatidylcholine. The PE and PS play important roles in the functionality of Ca^{2+} transport proteins, and the upregulation of PE, PS, and the Ca^{2+} transport proteins revealed there was an increase activity in Ca^{2+} re-sequestration of WB SR. Meanwhile, Ca^{2+} release and signaling proteins were downregulated, indicating attempts to control Ca^{2+} outflow from WB SR. So, what is the source of this elevated free Ca^{2+} in WB meat? The upregulation of AChE activity is a typical indicator of elevated AChE inhibitors as found in Alzheimer and Parkinson disease patients. Perhaps the inhibition of AChE extended the length of action potential and Ca^{2+} release from the SR. The upregulation of Ca^{2+} transport proteins and downregulation of Ca^{2+} release proteins in WB SR may be its attempt to regulate this proposed excessive signaling of Ca^{2+} release.

Keywords: calcium, lipidomics, proteomics, sarcoplasmic reticulum, woody breast