



## Apoptotic and Proteolytic Attributes and Metabolomic Changes in Postmortem Muscles from Pigs Subjected to Post-Weaning Transport at Different Seasons

D. Ma<sup>1\*</sup>, D. H. Suh<sup>2</sup>, J. Zhang<sup>1</sup>, A. W. Duttlinger<sup>3</sup>, J. Johnson<sup>3</sup>, C. H. Lee<sup>2</sup>, and Y. H. B. Kim<sup>1</sup>

<sup>1</sup>Department of Animal Sciences, Purdue University, West Lafayette, IN, USA

<sup>2</sup>Department of Bioscience and Biotechnology, Konkuk University, Seoul, Korea, Republic of

<sup>3</sup>USDA-ARS Livestock Behavior Research Unit, Purdue University, West Lafayette, IN, USA

\*Corresponding author. Email: ma128@purdue.edu (D. Ma)

**Keywords:** apoptosis, calpain, heat shock protein, proteolysis, seasonal metabolism  
Meat and Muscle Biology 3(2):166

### Objectives

Post-weaning transport of pigs was commonly practiced in the swine industry, however, adversely impact animal growth and well-being due to concurrent stress from weaning and transport. Further, our recent study found that post-weaning transport may have long-term effects on final pork quality attributes in terms of inferior texture and water-holding capacity. Heat shock proteins (HSPs) are anti-apoptotic chaperone proteins, protecting against apoptosis under a variety of cell death stimuli including postmortem muscle conversion process. While a potential role of apoptosis in meat tenderization has been proposed, how early life stress influences apoptotic/proteolytic process and metabolism of postmortem muscles is largely unknown. Thus, the study objective was to evaluate apoptotic and proteolytic attributes and metabolomic changes in postmortem muscles of market weight pigs exposed to early life transport/weaning stress at two seasons.

### Materials and Methods

Two repetitions of newly weaned pigs ( $N=480$ ) were transported for 12 h in a trailer truck during July 2016 (SUMMER) and April 2017 (SPRING) in north-central Indiana. Upon reaching market weight, 10 animals were randomly chosen from each season and slaughtered in January 2017 and September 2017, respectively. Pairs of *longissimus dorsi* and *psaos major* muscles from each carcass were separated at 1d and 7d postmortem. Proteolytic and apoptotic factors including desmin, troponin T, calpain 1, HSP27, and  $\alpha$ -crystallin were quantified using Western-blot assays, and mitochondria membrane permeability (MMP) was evaluated. Metabolome profiles of 1d samples were analyzed using the GC-TOF-MS/MS platform. Multivariate analyses PCA and PLS-DA were used to determine changes of metabolites. Data were analyzed

using PROC MIXED of SAS to compare the traits across season, muscle, and aging effects.

### Results

Previously, SUMMER pigs were reported showing decreased body weight, muscling, and fat deposition, as well as increased shear force and water loss during aging. In the present study, SPRING muscles exhibited increases in calpain 1 autolysis and structural protein degradation, coincided with accelerated apoptosis shown as higher MMP compared to the SUMMER counterparts ( $P < 0.05$ ). Moreover, PCA and PLS-DA clustering indicated distinct metabolome profiles affected by season and muscle. Seasonal effect mainly altered lipid, glucose, and nitrogen metabolism. A group of 16C to 18C fatty acids were increased in SPRING, probably due to increased lipid anabolism during warm growing/finishing season. Changes of urea, ornithine, aspartic acid, and 5'-methylthioadenosine suggested increased amino acid catabolism in SUMMER, corroborating the decreased lean and fat accretion. Seasonal changes of key metabolites related to stress response, including histidine, GABA, and ascorbic acid, suggested increased stress defense in SUMMER pigs, which implied the suppression of apoptotic and proteolytic activities.

### Conclusion

Taken together, SUMMER pigs showed suppressed onset of apoptosis with compromised growth and meat quality, possibly due to alternations in seasonal metabolic response. This may in turn affect the proteolytic potential of early postmortem muscles. Further studies elucidating the involvement of apoptotic process in proteolytic activities in postmortem muscles should be warranted.