INVOLVEMENT OF APOPTOSIS IN MEAT QUALITY ATTRIBUTES USING THE CALLIPYGE LAMB MODEL: 1. THE OMICS APPROACH

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Abstract –This study was conducted to characterize metabolome and proteome changes in *M. Longissimus* from callipyge mutation (+/C) and other non-callipyge phenotype (+/+, C/+, and C/C) lambs to provide insight into the biochemical changes of postmortem muscle and its impacts on meat quality characteristics. Apoptosis-related metabolites, such as sphingosine, and proteins including heat shock proteins and apoptotic factors, were found to be more abundant in the muscle from callipyge lambs compared to the muscles from other non-callipyge phenotype lambs. Our results suggest that meat toughness in the callipyge lamb may be attributed to the anti-apoptotic factors release and their subsequent impacts on postmortem proteolysis regulation.
Key Words – Proteomics Metabolomics Apoptosis

I. INTRODUCTION

The genetic mutation of callipyge sheep results in postnatal muscle hypertrophy in specific loin and hindquarter muscles. This phenotypic trait can only be observed in paternally inherited heterozygous (+/C; + = wildtype allele, C = mutant allele) lambs compared to other possible callipyge genotypes (+/+, C/+ and CC). The callipyge mutation is associated with the upregulation of calpastatin (inhibitor of calpain-1), which, in turn, adversely affects meat tenderness during postmortem aging. Besides the unique meat toughness, our recent publication found that loins from callipyge sheep had lower heme iron content and less discoloration during display compared to loins from non-callipyge phenotype lambs [1]. This is likely due to a shift of muscle fiber type toward fast-twitch, glycolytic muscle fiber in hypertrophied muscles [2], which leads to changes of muscle metabolism properties and protein expression that may relate to meat quality attributes. Omics techniques have been increasingly used to provide insight into the biochemical changes of postmortem muscle to discover the mechanisms by which postmortem processing can impact meat quality characteristics. Therefore, we aimed to further elucidate the molecular processes, apoptosis in particular, related to the muscle to meat conversion and subsequent meat quality attributes of the callipyge ovine muscles using proteomic and metabolomics approaches.

II. MATERIALS AND METHODS

A total of 16 loins (M. *longissimus thoracis*) from lamb carcasses of all four possible callipyge genotypes (+/C, C/+, C/C, and +/+; n = 4 each) was collected at 3 days postmortem and used for proteomics and metabolomics analyses. The extracted proteins were separated using a nanoLC system (Agilent), and scanned using a LTQ-Orbitrap mass spectrometer. WIFF files were processed using the MaxQuant computational proteomics platform at minimum peptide length of seven amino acids and were analyzed using UniProtKB Bos taurus and/or Ovis aries databases. For metabolomics analysis, protein removal and sample extraction were performed according to Bligh-Dyer method. The polar and non-polar phases were separated on an Agilent 1100 system, and was scanned using an MSD-TOF spectrometer attached with ESI capillary. Mass data (from m/z 70-1100) were collected using Agilent MassHunter software. All the data were analyzed using PROC MIXED procedure of SAS with genotype as a fixed effect and animal as a random effect. The significance threshold ($P < 0.05$) was corrected using Bonferroni correction and Benjamin-Hochberg's false discovery rate (FDR) adjustment.

III. RESULTS AND DISCUSSION

Metabolites that were significantly affected by the genotypic effect included small peptides and dietary derived phenolic compounds (Table 1; selected metabolites). Two sphingosine related metabolites, dehydro-phytosphingosine and sphingosine kinase (SK) inhibitor 2, were affected by the genotype, where sphingosine kinase inhibitor 2 was more abundant in the callipyge group (+/C). The sphingosine metabolites mediate several major molecular and signaling

pathways that determine cell fate such as survival and apoptosis. In addition, enhancement of SK has known to activate anti-apoptotic factor HSP27[3].

Table 1

Mass_ Input	Putative metabolites	Highest in	Lowest in	Pr
Amino acids and dipeptides				
214.1294	Pro-Val	C/+	+/C	0.03
240.1155	L-Anserine	+/C	C/+	0.04
296.1198	Met-Phe	+/C	CC	0.04
188.1244	Homoarginine	+/C	C/+	0.03
dietary derived phenolics and other secondary metabolites				
168.0788	2,6-Dimethoxy-4-methylphenol	+/+	CC	0.01
208.1448	Heptyloxyphenol	+/+	C/+	0.02
234.1601	Macrophyllic acid	+/+	C/+	<0.01
542.1371	Resveratrol 4'-(2-galloylglucoside)	+/C	+/+	<0.01
Primary metabolites				
315.2756	Dehydro-phytosphingosine	+/+	C/+	<0.01
302.0154	Sphingosine Kinase Inhibitor 2	+/C	C/+	<0.01
348.0435	IMP	+/C	C/+	0.01
245.1638	Pivaloylcarnitine	+/+	+/C	0.02

The proteomics analysis identified 307 proteins that were significantly affected by the genotype (Table 2; selected proteins). Most proteins are related to glycolytic and/or oxidative energy metabolism, muscle structure, and function. In general, glycolysis related enzymes were upregulated in the +/C (callipyge) group, while proteins that were related to mitochondrial function or oxidative pathways, such as SOD, were down regulated in the callipyge group (Table 2). In addition, a group of anti-stress/anti-apoptotic related proteins were found to be more abundant in the +/C animals, including BCL2 inhibitor, protein DJ, and heat shock proteins (Table 2). In our subsequent study, the Western-blot analyses of the same samples showed that loins from the callipyge group exhibited greater intact HSP27 and procaspase-3 along with high calpastatin and more intact calpain-1 subunits compared to the loins from non-callipyge phenotype lambs [4]. The current proteomics results are in agreement with these findings and support the postulation that upper stream apoptotic factors (e.g. BCL2) and molecular chaperons maybe involved in regulating postmortem proteolysis and the subsequent tenderization process.

Table 2

UNIPro ID	Protein name	Highest in	Lowest in	Pr
Energy metabolism related				
W5P1X9	Fructose-bisphosphate aldolase	+/C		0.05
W5QFQ0	Malate dehydrogenase	+/C		0.05
W5PFT7	Fructose-bisphosphatase 2	+/C		0.05
A5D984	Pyruvate kinase	+/C		0.03
Q08DP0	Phosphoglucomutase	+/C		0.03
W5PVY5	Phosphoglycerate mutase 2	+/C		0.05
W5PIG7	Enolase	+/C		0.03
Q29RZ0	Acetyl-CoA acetyltransferase;		+/C	0.04
W5P340	Superoxide dismutase		+/C	0.04
Structural, regulatory, contractile and other muscle functions				
W5Q0I1	myosin binding protein C, slow type		+/C	0.05
M5FI55	Sarcoplasmic reticulum glycoprotein-like	+/+	+/C	0.05
W5QG29	Desmin	CC	+/+	0.01
Other functional and transcript proteins				
W5PG95	HSP70	+/C		0.05
Q3T149	HSP27	+/C		0.01
W5PK66	Protein DJ	+/C		0.01
W5NYH4	BCL2 associated athanogene 3	+/C		0.01
A7E3S8	HSP70 binding protein	+/C		0.05

IV. CONCLUSION

The results of the current study found that several novel factors and pathways, including sphingosine metabolites, BCL2, protein DJ, and heat shock proteins, were significantly affected by the callipyge genotypes. Taken together, it would be reasonable to postulate that meat toughness, the well-known phenotypic trait of the callipyge lamb, would be likely attributed to the anti-apoptotic factors and their subsequent impacts on postmortem proteolysis regulation. Further studies elaborating the exact role of heat shock proteins in the myofibrillar protein degradation of postmortem muscles should be warranted.

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