2019 Reciprocal Meat Conference – Muscle and Lipid Biology and Biochemistry

Meat and Muscle Biology™

# Differential Protein Abundance in Dark-Cutting and Normal-pH Beef

F. Kiyimba[1]*, S. Hartson[2], J. Rogers[2], G. Mafi[1], D. VanOverbeke[1], and R. Ramanathan[1]

[1]Animal & Food Sciences, Oklahoma State University, Stillwater, OK, USA
[2]Biochemistry & Molecular Biology, Oklahoma State University, Stillwater, OK, USA
*Corresponding author. Email: frank.kiyimba@okstate.edu (F. Kiyimba)

**Keywords:** dark-cutting, mass spectrometry, meat color, proteomics
Meat and Muscle Biology 3(2):163

## Objectives

Dark-cutting beef is a meat quality defect in which meat does not display the marketable bright-red color. Although previous studies have indicated that the ultimate pH of dark-cutting beef is greater than normal, the mechanistic basis for the occurrence is not clear. Various mitochondrial and glycolytic enzymes/proteins are involved in muscle metabolism and lowering of pH. However, limited knowledge is currently available on the muscle protein profile differences between dark-cutting and normal-pH beef. The objective of the current study was to identify proteins related to the development of the dark-cutting condition by comparing the protein expression differences between dark-cutting and normal-pH beef.

## Materials and Methods

Dark-cutting and normal-pH beef samples were collected from six ($n$ = 6) different animals after slaughter. Tissue samples (0.5 g) were digested in 5 mL of lysis buffer. Tissue lysates were homogenized, boiled, sonicated using a bioruptor and centrifuged at 10,000 g for 10 min. Samples were digested with trypsin/Lys-C overnight at 37°C, after which additional 2 µg/mL of protease was added and digestion was continued for another 8h. The resulting trypsinolytic peptides were acidified to 1% trifluoroacetic acid and purified by solid phase extraction with C18 affinity media. Protein expression profiles of both dark-cutting and normal-pH beef samples were determined using LC-MS/MS mass spectrometry-based proteomics. Collected raw data instrument files were searched against a bovine proteome database of 23,968 bovine proteome sequences using MaxQuant (V.1.5.3.8). Differential protein expression analysis was done in Perseus (V.1.5.1.3). Ingenuity pathway analysis (IPA) was utilized to determine the significant pathways of the differentially expressed proteins in dark-cutting and normal-pH beef. Gene ontology enrichment pathway analysis was performed to determine the main functions of the differentially expressed proteins in dark-cutting and normal-pH beef identified in our samples.

## Results

Mass spectrometry analysis identified 1148 proteins, and 97 of these proteins were differentially expressed between normal-pH and dark-cutting beef ($P < 0.05$). Fold change of 1.5 was observed for 29 proteins. Dark-cutting beef had 19 abundant proteins, while normal-pH beef had 10 abundant proteins. The majority of the upregulated proteins in dark-cutting beef were involved in mitochondrial functioning and metabolism, while the majority of the downregulated proteins were important in glycogen degradation, calcium signaling, α-adrenergic signaling, n-NOS-signaling and the proteasome pathways.

## Conclusion

The results identify new protein biomarkers associated with dark-cutting and suggest new mechanistic explanations for the dark-cutting phenotype.