Dietary supplementation of vitamin E influences myoglobin post- translational modifications in beef longissimus lumborum

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Objective: Fresh meat discoloration is due to the oxidation of myoglobin and adversely affects consumer perception of quality. The secondary products of lipid oxidation can accelerate myoglobin oxidation through alkylation of myoglobin leading to meat discol- oration. Post-translational modifications (PTM) in myoglobin influence fresh beef color stability. Dietary supplementation of vita- min E improves beef color stability by delaying lipid oxidation-induced myoglobin oxidation, and the effect of vitamin E on fresh beef color have been extensively studied from the standpoint of lipid oxidation-induced myoglobin oxidation. Furthermore, dietary supplementation of vitamin E influenced the mitochondrial and sarcoplasmic proteome profiles of postmortem beef longissimus lumborum muscle. Nonetheless, the influence of vitamin E on myoglobin PTM in post-mortem beef skeletal muscles has yet to be investigated. Therefore, the objective of this study was to examine the effect of dietary vitamin E on myoglobin PTM in post-mor- tem beef longissimus lumborum muscle.

Materials and Methods: Beef longissimus lumborum muscle samples (24 hours post-mortem) were obtained from the carcasses of nine (n = 9) vitamin E-supplemented (VITE; 1000 IU vitamin E diet/heifer·d-1 for 89 days) and nine (n = 9) control (CONT; no supplemental vitamin E) heifers. Sodium dodecyl sulfatepolyacrylamide gel electrophoresis was used to separate myoglobin from other sarcoplasmic proteins of beef longissimus lumborum muscle. The protein band (17 kDa) representing myoglobin in the gels were excised and subjected to liquid chromatography -electrospray ionization-tandem mass spectrometry. Mass spectrometric data were analyzed via Proteome Discoverer for identification of myoglobin PTM such as: methionine oxidation; lysine acetylation; ly- sine mono-, di-, and tri-methylation; arginine mono- and dimethylation; lysine carboxymethylation; serine, threonine and tyrosine phosphorylation; 4-hydroxynonenal (HNE) alkylation at histidine, and lysine.

Results: Tandem mass spectrometry identified multiple PTM (phosphorylation, acetylation, alkylation, methylation, dimethylation, trimethylation, and carboxymethylation) in myoglobin. The amino acids susceptible to phosphorylation were threonine (T) and tyrosine (Y), whereas lysine (K) residues were prone to other PTM. A greater number of amino acids were modified in CONT than VITE (16 vs 13). Myoglobin from CONT and VITE demonstrated similar pattern in phosphorylation (T34, T67, Y103), carboxy- methylation (K77, K78), and HNE alkylation (K77, K78, K79) sites, suggesting these PTM were not significantly influenced by the vitamin E supplementation in beef animals. Nonetheless, differential occurrence of acetylation, methylation, dimethylation and trimethylation were identified in myoglobin from CONT and VITE samples. Acetylation at K87 and methylation at K98 were unique to CONT, whereas acetylation and methylation at K118 were unique to VITE. Dimethylation at K118 and K133 were only detected in VITE myoglobin. While K96, K102 and K133 were tri-methylated in CONT, only K118 were trimethylated in VITE. Overall, the unique PTM in CONT myoglobin were spatially closer to proximal histidine compared to the unique PTM in myoglo- bin from VITE samples, and thus could compromise myoglobin redox stability due to the potential interference with proximal histidine.

Conclusion: Dietary supplementation of vitamin E decreased the numbers of post-translationally modified residues in myoglobin from beef longissimus lumborum. While phosphorylation, carboxymethylation, and alkylation were not influenced by vitamin E supplementation, differential acetylation, methylation, dimethylation and trimethylation sites were identified in myoglobin from CONT and VITE beef samples. The unique PTM in CONT myoglobin (K87, K96, K98 and K102) were spatially closer to proximal histidine compared to the unique PTM (K118) in myoglobin from VITE samples, and thus could compromise myoglobin redox stability due to the potential interference with proximal histidine. Dietary supplementation of vitamin E in beef cattle might protect residues in myoglobin, especially those located spatially close to proximal histidine, from undergoing PTM, and thereby improving myoglobin redox stability.

Key words: Myoglobin, Post-translational modifications, Vitamin E