METABOLOMIC INVESTIGATION OF TENDERNESS AND AGING RESPONSE IN BEEF LONGISSIMUS STEAKS

D. Andy King[1*], Steven D. Shackelford[1], Corey D. Broeckling[2], Jessica E. Prenni[2], Keith E. Belk[3], and Tommy L. Wheeler[1]

[1]USDA-ARS-US Meat Animal Research Center, Clay Center, NE, 68933, USA;

[2]Proteomics and Metabolomics Facility, Colorado State University, Fort Collins, CO, USA

[3]Department of Animal Science, Colorado State University, Fort Collins, CO, USA

*Corresponding author email: andy.king@ars.usda.gov

I. INTRODUCTION

Substantial investment has been made in understanding biochemical factors affecting beef tenderness. Component traits such as sarcomere length and postmortem proteolysis have been used to expand understanding of mechanisms responsible for animal variation in beef tenderness and the extent of the aging response. However, component traits explain a limited amount of the variation in tenderness, and sometimes fail to characterize tenderness differences. Thus, the present experiment was conducted to identify metabolomic biomarkers explaining differences in beef tenderness and potential relationships between these biomarkers and sarcomere length and desmin degradation.

II. MATERIALS AND METHODS

Carcasses (U.S. Select: n = 30) were selected to represent extremes in tenderness using the tenderness prediction model in the VBG2000GigE beef grading system. Beef, loin, strip loin subprimals were obtained from the left side of each carcass. Steaks from each of the anterior and posterior halves of the longissimus lumborum, 4 pairs of adjacent steaks were blocked by location and assigned to be aged until 2, 7, 14, or 28 days postmortem. Thus, two pairs of steaks from each strip loin were assigned to each aging time. One steak from each pair was designated for slice shear force determination and the other was designated for biochemical analysis. Slice shear force was accomplished using an established protocol[1]. Steaks designated for biochemical analysis were used for sarcomere length determination[2], quantification of desmin degradation[3], and non-targeted LC and GC/MS metabolite profiling[4].

Slice shear force values from all aging times were utilized to identify the carcasses with the lowest (most tender) and highest (toughest) slice shear force values (n = 10 per group). Analysis of variance and correlation analysis were used to identify metabolites that were strongly related (P-values exceeding 2×10^{-5}) to slice shear force, desmin degradation, and sarcomere length. Metabolites exceeding these criteria were included in partial-least squares regression analysis with slice shear force, desmin degradation, and sarcomere length as dependent variables, and tenderness class and aging time as fixed effects.

III. RESULTS AND DISCUSSION

Steaks classified as tough had higher ($P < 0.001$) slice shear force values than steaks classified as tender. Increasing aging time decreased ($P < 0.001$) slice shear force values. Steaks from carcasses classified as tender on d 2 postmortem had slice shear force values similar ($P = 0.42$) to those of steaks from carcasses classified as tough on d 28 postmortem. Steaks classified as tender had a greater ($P<10^{-4}$) proportion of desmin degraded than steaks from carcasses classified as tough. Increasing aging time increased ($P < 10^{-22}$) the amount of desmin degraded in steaks from both tenderness classes. Sarcomere length did not differ across tenderness classes or aging times.

A total of 113 metabolites with P-values exceeding the Bonferroni correction level ($P < 2 \times 10^{-5}$) in analysis of variance and correlation analyses were included in further analyses. No metabolites met this level of significance for correlation to sarcomere length, so an additional 6 metabolites were included based on partial correlations significant at ($P < 0.01$). Thirty-six metabolites that met the screening criteria could be annotated and some were loosely categorized into amino acids/peptides (n = 16), metabolism intermediates (n = 7), glycosides (n=4), fatty acids and phospholipids (n =3).

Each of the amino acids increased ($P < 10^{-5}$) with increased aging time and generally were present in greater ($P < 0.05$) levels in steaks classified as tender. Metabolites classified as glycosides increased ($P < 10^{-5}$) with increased aging time. Levels of three compounds with masses suggestive of plant glycosides were greater ($P < 10^{-4}$) in steaks classified as tender, while levels of one were greater ($P=10^{-4}$) in steaks classified as tough. Levels of free glucose and glucose-6-phosphate were greater ($P< 0.03$) in steaks classified as tender than in steaks classified as tough, while levels of malic acid and glycerol-3-phosphate were greater in steaks classified as tough. Levels of free glucose increased ($P< 10^{-10}$) during aging, while glucose-6-phosphate, malic acid, and glycerol-3-phosphate levels decreased ($P < 10^{-6}$) with increased aging time.

The first two factors derived in the PLS analysis explained 40 and 10% of the variance in the dependent variables, respectively. The first factor was highly related to both increased slice shear (positive values) force and increased desmin degradation (negative values). Aging time was strongly associated with Factor 1 with day 2 postmortem associated with positive values and increased aging times being associated with lower and negative values of Factor 1. The tender class was also associated with negative values for Factor 1, while the tough class of steaks was associated with positive values. The second factor was highly associated with the tenderness classes with the tough class along with increased slice shear force being associated with positive values and the tender classes being associated with negative values.

Amino acids were primarily related to negative values for factor 1, and clustered around the mapping for desmin degradation. Increased glucose levels were strongly related to the tender classification and moderately related to increased proteolysis, while increased glucose-6-phosphate was strongly related to the tender class, but was related to decreased proteolysis. Increased malic acid was strongly related to the tough classification, increased slice shear force, and decreased proteolysis. Increased levels of glycerol-3-phosphate was moderately associated with increased slice shear force and decreased proteolysis. Increased glycoside levels were strongly related to negative values for Factor 1 and primarily clustered around the mapping for postmortem proteolysis.

IV. CONCLUSION

These data indicate that accumulation of amino acids during aging is strongly related to postmortem proteolysis and may provide evidence of the fate of proteins degraded postmortem. Measures of glucose, glucose-6-phosphate, and malic acid concentrations may provide a metabolic fingerprint indicative of tenderness differences in beef longissimus. Further study into the role of glycosides in regulating tenderness variation in beef longissimus is warranted. These data indicate further evaluation of the association of muscle metabolites to measures of meat tenderness should be pursued.

ACKNOWLEDGEMENTS

The authors thank Patty Beska, Kristin Ostdiek, Casey Trambly, and Peg Ekeren for technical support and Jody Gallagher for secretarial assistance.

REFERENCES

1. Shackelford, S.D.,. Wheeler, T.L, and Koohmaraie, M. (1999) Evaluation of slice shear force as an objective method of assessing beef longissimus tenderness. Journal of Animal Science 77(10): 2693-9.
2. Cross, H.R., West, R.L., and Dutson, T.R. (1981). Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. Meat Science 5: 261-6.
3. Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. (2002). Technical Note: Sampling methodology for relating sarcomere length, collagen concentration, and the extent of postmortem proteolysis to beef and pork longissimus tenderness. Journal of Animal Science 80: 982-987.
4. Turner, M.F., Hueberger, A.L., Kirkwood, J.S., Collins, C.C., Wolfrum, E.J., Broeckling, C.D., J.E. Prenni, and C.E. Jahn. (2016). Non-targeted Metabolomics in Diverse Sorghum Breeding Lines Indicates Primary and Secondary Metabolite Profiles Are Associated with Plant Biomass Accumulation and Photosynthesis. Frontiers in Plant Science 7(953).