Subgroup 2

Enzymes and proteins

THE SIGNIFICANCE OF THE 30 kDa POLYPEPTIDE IN PREDICTING TENDERNESS OF SIMILAR AGED POST MORTEM TISSUE

L. Basu[1], M. P. Wick[1], H. Ockerman[1], M. Yamaguchi[2] and D. M. Wulf[3]
[1]Dept. Of Animal Sciences, The Ohio State University, Columbus, Ohio 43210, USA
[2]Dept of Veterinary Bio Sciences, The Ohio State University, Columbus, Ohio 43210, USA
[3]Dept of Animal and Range Science, South Dakota State University, SD 57007, USA

Background

Tenderness is the primary sensory attribute influencing the acceptability of beef. Myofibrillar protein degradation plays a predominant role in determining tenderness in skeletal muscle. The mechanisms controlling muscle tenderness are not well understood. In spite of the current U.S quality grading system, tenderness is not always assured and uniform, animals of the same age, treated and fed similarly, under similar management conditions often result in meat that is different in tenderness. Due to this variability and lack of reproducible animal models, little information is available on reliable predictors of meat tenderness. The aim of this study was to identify the similarities and differences in the myofibrillar protein patterns in the *Longissimus lumborum* muscle of postmortem age matched beef carcasses.

Objectives

The objective of this research was to determine the contribution of myofibrillar protein patterns to ascertain muscle tenderness in postmortem age matched beef carcasses. These measures of protein degradation may be important predictors of muscle tenderness and contribute to our understanding of the role of myofibrillar protein architecture in determining tenderness

Methods

Sampling, Sensory Evaluation, Shear Force: Beef carcasses (n=1,062) were selected to match the breed type, sex class, marbling score, dark cutting discount, overall maturity, hot carcass weight and yield grade distribution as reported in the 1995 US, National Beef Audit. Of these carcasses 100 were selected to represent the three breed types and analyzed for USDA grade measurements, muscle color, pH, temperature, and electrical impedance of the *L lumborum* (LL) muscle. Samples aged 7 d PM were evaluated for tenderness by a trained sensory panel and Warner Bratzler shear. Based on these results 10 carcasses, 5 tough and 5 tender were selected for further analysis. (Table 1)
Myofibril preparation: Beef *Longissimus lumborum* and rabbit Psoas (reference standard) myofibrils were prepared, (Yamaguchi et al., 1975). Protein concentrations were determined by Biuret method with a Beckman spectrophotometer (UV/Vis, Model 35). Minislab SDS/PAGE was performed using 4-20% pre-cast gradient gel (Novex, San Diego).
Histological Evaluation: The suspended myofibril from both extremes of tough and tender were viewed under a light microscope (Zeiss photomicroscope III). One sample was selected for Transmission electron microscopy. Sections doubly stained with uranyl acetate and lead citrate were examined under a Hitachi H-300 Electron Microscope at 75 kV.
Immunoblotting: 10% SDS/PAGE slab gels were transferred to a nitrocellulose membrane using a Minni-genie Blotter (Idea scientific Co., Minneapolis). The membrane was incubated with mouse monoclonal anti-troponin T, dilution 1:100, (clone JLT-12, Sigma Chemical Company, St. Louis). Bound primary antibody were labeled with goat antimouse IgG horseradish peroxidase conjugated secondary antibody (Kirkengand Pery, Gaithersburg). Freshly prepared rabbit psoas was utilized as a reference standard.
Laser densitometry: Density was determined by Bio-Rad GS-700 Imaging densitometer and the bands were analyzed using Image Pro analysis software.
Data Analysis: Data was analyzed by one - way Analysis of Variance. Multiple comparisons were performed utilizing general linear models.

Results and Discussion

Histological examination of myofibrillar extraction: Light microscopic examination showed that the tender myofibrils of animals of the same degree of aging were more fragmented than the tough myofibrils. The fragments of the tender myofibrils were shorter in length and less clumping leading to the formation of aggregates was noted. Electron microscopic analysis of the myofibrillar pellet again showed much more fragmentation of the tender tissue. Less muscle structural organization was observed in the tender tissue.
Myofibrillar protein activity: The effect of a ~30 k Da degradation band was most pronounced in the tender tissue as noted visually (Figure 1). One way analysis of variance was used to analyze the effect of ~ 30 k Da degradation band versus shear force and sensory panel. The 30 k Da band from the tender and tough samples were analyzed by analysis of variance. There was a highly significant ($p = 0.01$) difference in the quantity of the ~ 30 kDa degradation band which was more abundant in the tender tissue.
Western blot analysis: Western blot analysis indicated at least three degradation bands of troponin T that appeared on both tender and tough samples, in the ~30 kDa area. The predominant band ~ 32 was more abundant in the tender samples which suggested that the ~30 kDa noted previously in predominately tender tissue is a degradation product of troponin T (Figure 2).

Conclusions

The quantity of the ~30 kDa unidentified degradation band data was compared to the Warner Bratzler shear values and taste panel tenderness. For the two measurements of tenderness, the Warner Bratzler shear and the sensory panel the correlation values when compared to the quantity of the ~30 kDa degradation band values were highly significant (Warner Bratzler shear, $p = 0.01$; sensory panel tenderness, $p = 0.01$). The analysis of variance of the regression equation was also highly significant (Warner Bratzler shear, $p= 0.000$; sensory panel tenderness, $p= 0.000$).
This suggest that the quantity of the ~30 kDa degradation band could be successfully used as an indicator of tenderness (R^2 values for Warner Bratzler shear =0.71, sensory panel tenderness = 0.63 of animals that were similarly aged.
Sodium dodecyl sulfate gel electrophoresis showed differences in myofibrillar protein patterns between tough and tender muscles. Visual comparisons indicated differences in Troponin T degradation and was confirmed using western blot analysis. Weakening of the myofibrillar structure was further demonstrated by ultrastructural comparisons using light and transmission electron microscopy. All the tender samples of similarly aged animals had a 30 to 32 kDa band that was either invisible or very faint in all the tough samples. The degradation of Troponin T resulting in ~30 kDa polypeptide could weaken the thin filament. It therefore suggests that the presence or absence of this troponin T degradation product (~30 kDa) could be a useful tool in predicting tenderness of similar aged postmortem tissue.

Pertinent Literature

Asghar, A. and Bhatti, A. R. Jr. 1987. Collagenous proteolytic enzymes in skeletal muscle: Their significance in muscle physiology and during postmortem aging events in the carcasses. Adv. Food Res. 31,343-445.

Greaser, M. L. 1986. Conversion of muscle to meat. In: Muscle as Food. Bethehel, P. J. (Ed) Academic Press. London. p 37-102.

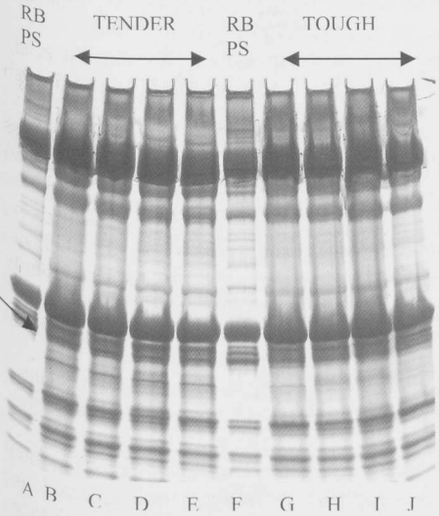
Ho, C. Y., Stromer, M. H., and Robson R. M. 1994. Identification of the 30 kDa polypeptide in post mortem skeletal muscle as a degradation product of troponin T. Biochimie, 76, 369-375.

Koohmaraie, M. 1988. The role of endogenous proteases in meat tenderness. Proc. Of Recip. Meat Confierence. 41, 89-100.

Yamaguchi, M., Cassernsl, R., and Dahl, D. 1975. Congenital rod disease: some biochemical aspects of nemaline rods. Cytobiologie 11, 335-345.

Table 1. Shear and Tenderness scores on the *Longissimus lumborum* used to separate carcasses into Tough and Tender categories

Carcass Identification	Age Postmortem	Warner Bratzler Shear Value	Panel Tenderness	Sensory Panel Juiciness
Tender (34)	7 days	2.62	7.455	6.591
Tender (45)	7 days	2.10	7.08	5.966
Tender (47)	7 days	2.42	7.667	6.222
Tender (74)	7 days	2.32	7.556	5.778
Tough (19)	7 days	6.97	4.444	6.556
Tough (35)	7 days	7.08	2.889	5.556
Tough (68)	7 days	8.80	2.222	5.667
Tough (76)	7 days	7.43	3.778	4.889



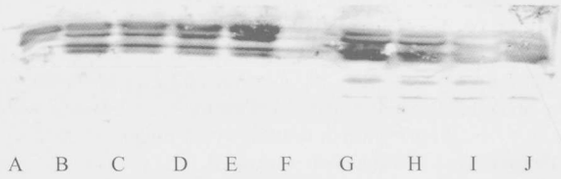


Figure 2 Western Blot analysis of myofibrillar protein patterns of tough and tender carcasses.

Lane A represents rabbit psoas myofibril control. Lane F represents the low molecular wt markers. The predominant band is located in the ~ 30 kDa area and is represented in Lane (G, H, I, J), indicated as tender myofibrillar protein lane. **Note** this band represents the troponin T degradation product stained with monoclonal rabbit anti-troponin T.

Figure 1 SDS polyacrylamide gel pattern of bovine *Longissimus lumborum* myofibrillar protein separated on 4-20% gradient gel.

Lane (A & F) show rabbit psoas myofibril used as a control. Lane (B, C, D, E) represent the tender samples. Lane (G, H, I, J) represents the tough samples. **Note** a significant ~ 30 kDa band is observed in the tender myofibrillar protein pattern.