RELATIONSHIP BETWEEN MUSCLE MICROSTRUCTURE, CALPASTATIN ACTIVITY AND SHEAR FORCE IN AGED *LONGISSIMUS DORSI* MUSCLE

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Key Words: beef, calpastatin, microstructure, tenderness, Warner-Bratzler shear force

Introduction

Tenderness is a critical factor determining the consumer's acceptance of meat. However significant variations in the tenderness of beef can be found at the retail and food service levels, and the unpredictable nature of this variation can have serious economic impacts on the meat industry. It is now well established that meat tenderness improves during *postmortem* cooler storage and that this tenderisation process mainly results from the calpain-mediated proteolysis of key myofibrillar and associated proteins (for reviews, see Goll et al., 1991; Koohmaraie, 1996).

This proteolysis does not appear to be random, and several studies have been undertaken to determine which specific proteins are degraded during *postmortem* storage of meat and what microstructure changes are associated with tenderisation. Taylor et al. (1995) showed that degradation of costameres, intermediate filaments, titin and nebulin are likely to be responsible for *postmortem* tenderisation of meat. The main function of these proteins is to maintain the structural integrity of the myofibrils. Once degraded, the rigid structure of the myofibrils is weakened leading to breakage and more tender meat. Another structural change seen during *postmortem* storage is detachment of the endomysium from the muscle fibres, probably due to degradation of proteoglycans in the extracellular matrix (Hannesson et al., 2003).

The objective of this study was to investigate the relationships between microstructural changes, calpastatin activity, and Warner-Bratzler shear force (WBSF) in aged *Longissimus dorsi* muscle from Norwegian Red Cattle.

Materials and methods

Fifty Norwegian Red bulls (12-14 months of age) were slaughtered in nine batches over a 3-year period. Bulls were transported (1h) to a commercial abattoir and slaughtered upon arrival. Carcasses were electrically stimulated (90V) 20 minutes after exsanguination, and the *Longissimus dorsi* (LD) muscle was removed from the 10th thoracic to the 2nd lumbar vertebrae. The LD portions were vacuum-packed and conditioned at 12˚C for 10 h before ageing for 7 days at 4˚C.

Samples (n=36) for calpastatin were extracted between 24 and 31 hrs *postmortem* using the heated calpastatin procedure of Shackelford et al. (1994). Following extraction and heat treatment, calpastatin activity was determined using BODIPY-FL labelled casein according to Thompson et al. (2000). At 7 days, samples (n=50) for WBSF (3.5 cm thick) were vacuum packed, heated in a water bath at 70°C for 50 min and chilled in iced water for 45 min. Portions (1cm thick) were then cut from the cooked meat with slices parallel to the fibre direction. This produced 10 samples (2x1x1cm) which were sheared using a WBSF triangular version device mounted in an Instron Materials Testing Machine.

For microscopy, muscle blocks (2 x2x3mm) from the aged (7 day) samples (n=50) were fixed with 2.5% glutaraldehyde in cacodylate buffer and embedded in plastic resin as previously described (Ofstad et al., 1993). For all samples, 3µm sections were cut both perpendicular and longitudinal to the fibres. Optical microscopy was performed on sections stained in 0.1 g/100 ml toluidine blue dissolved in 0.1M sodium acetate, with a 20x objective to measure myofibre-to-myofibre detachment (MM-d) and myofibre-to-perimysium detachment (MP-d). Quantification of structural changes was performed as previously described by Taylor and Frylinck (2003). For MM-d this involved counting the numbers of myofibres attached versus partly or completely detached from the neighbouring myofibres for a minimum of 200 myofibre-to-myofibre attachments on transversally sectioned samples. MP-d was determined in a similar manner by counting the number of the myofibres attached and partly or completely detached from the perimysium for a minimum of 30 myofibre-to-perimysium attachments for each sample. Finally, contracted and broken myofibres (partly and totally) were counted on no fewer than 30 fibres on longitudinally sectioned samples.

Before correlation analyses (SAS, 1999), the data were corrected for the fixed effects of age, batch and pen within batch.

Results and discussion

It is well established that proteolysis of key myofibrillar and cytoskeletal proteins leads to increased fragmentation of myofibrils during *postmortem* storage of meat (Taylor and Koohmaraie, 1998; Ho et al., 1996). However, most of the quantitative changes previously described have been at the ultrastructural level (i.e. fractured myofibrils), while in this study we have focused on changes occurring at the microstructural level.

The correlation analyses of our data show a significant positive correlation between calpastatin levels and WBSF (Table 1), which is in agreement with previous reports (Shackelford et al., 1994). Moreover, we found significant negative correlations between myofibre breaks and both WBSF and calpastatin activity (Table 1). Thus, the myofibre breaks are probably caused by reduced calpastatin activity, which allows higher calpain activity resulting in increase proteolysis and improved meat tenderness (Taylor and Koohmaraie, 1998). In relation to the other microstructural features investigated in this study (i.e. MM-d, MP-d, and contracted myofibres), no significant correlations with WBSF or calpastatin activity were found, indicating that these changes do not play an important role in determining beef tenderness as measured with WBSF in these animals.

Table 1. Correlations between microstructural data, calpastatin activity, and Warner-Bratzler shear force (WBSF) on bovine M. *longissimus dorsi* stored for 7 days

Measurements	WBSF	Calpastatin
Calpastatin	0.62 (P<0.0001)	
Broken myofibres	-0.33 (P=0.02)	-0.55 (P<0.01)
MM-d	0.12	0.18
MP-d	-0.19	0.00
Contracted myofibres	-0.08	-0.03

Conclusions

The results of this study show that myofibre breaks is the main structural feature responsible for variation in WBSF of bovine LD muscle after cooler storage for 7 days *postmortem*. Moreover, the results also indicate that myofibre breaks are resulting from calpain-mediated proteolysis. Changes in myofibre-to-myofibre and myofibre-to-perimysium detachment seem to have little impact on beef tenderness.

References

Goll, D.E., Taylor R.G., Christiansen J.A., and Thompson V.F. (1991), Role of proteinases and protein turnover in muscle growth and meat quality. *Proceeding of the 44th Annual Reciprocal Meat Conference*, Manhattan, KS, 25-33.

Hannesson, K.O., Pedersen, M.E., Ofstad, R., and Kolset, S.O. (2003), Breakdown of large proteoglycans in bovine intramuscular connective tissue early postmortem. *Journal of Muscle Foods, 14*, 301-318.

Ho, C.Y., Stromer, M.H., Robson, R.M. (1996), Effect of electrical stimulation on post mortem titin, nebulin, desmin, and troponin-Y degradation and ultrastructural changes in bovine longissimus muscle. *Journal of Animal Science, 74*, 1563-1575.

Koohmaraie, M. (1996), Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Science, 43*, 193-201.

Ofstad R., Kidman, S., Myklebust R., Olsen, R.L., and Hermansson, A.M. (1993), Liquid holding capacity and structural changes during heating of fish muscle, cod (gadhus morhua L.) and salmon (Salmo salar). *Food Structure, 12*, 163-174.

Shackelford, S.D., Koohmaraie, M., Cundiff, L.V., Gregory, K.E., Rohrer, G.A., and Savell, J.W. (1994), Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner-Bratzler shear force, retail product yield and growth rate. *Journal of Animal Science, 72*, 857-863.

Taylor, R. and Frylinck, L. (2003), Muscle structures which determine meat tenderness in South African and other beef breeds. *Proceedings of the 11th International Meat Symposium*, 23-27.

Taylor, R.G., Geesink, G.H., Thompson, V.F., Koohmaraie, M., and Goll, D.E. (1995), Is Z-Disk Degradation Responsible for Postmortem Tenderization. *Journal of Animal Science, 73*, 1351-1367.

Taylor, R.G. and Koohmaraie, M. (1998), Effects of postmortem storage on the ultrastructure of the endomysium and myofibrils in normal and callipyge longissimus. *Journal of Animal Science, 76*, 2811-2817.

Thompson, V.F., Sladaña, S., Cong, J., and D.E. Goll. (2000), A BODIPY fluorescent microplate assay for measuring activity of calpains and other proteases. *Analytical Biochemistry, 279*, 170-178.