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VARIATION IN MUSCLE MICROSTRUCTURE AND BEEF TENDERNESS IN AGED *M. LONGISSIMUS DORSI* OF NORWEGIAN RED CATTLE (NRF) - PRELIMINARY RESULTS

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Introduction

Muscle is a composite structure comprised of contractile myofibres attached by connective tissue. Post mortem storage of meat is known to increase tenderness, i.e. being related to the degree of alterations of the structural components of the muscle and associated proteins during rigor and subsequent post rigor storage. Structural changes which occur post mortem are fibre contraction, breaks in the cytoskeleton proteins and thereby weakening of I-band near the z-disk with subsequent breaks in the sarcomeres (Taylor et al., 1995). Furthermore, detachment of the endomysium occurs, probably due to degradation of the proteoglycans in the extracellular matrix (Hannesson et al., 2003)). PH fall and calpain mediated proteolyses are furthermore regarded as important contributors to the structural alterations (Koohmaraie, 1996).

Less clear is how the variability of muscle morphology and composition affect the post mortem structural alterations and thereby quality. Shackelford et al. (1994) found that variation in calpastatin not always explain all tenderness phenomena. Sometimes other factors such as connective tissue characteristics and muscle contraction also play a role in tenderness of the aged meat. Frylinck and Heinze (2003) showed that genotypic meat quality differed among some African breeds due to genetically determined interacting biochemical and physiological factors. Fibre contraction, fibre-fibre attachment, and fibre breaks were significantly effected by breed-ageing, and also correlated to shear force and protease activity (Taylor and Frylinck, 2003). It has been shown thatpart of the variation in tenderness is inherited in Norwegian Red Cattle (NRF). In a pilot study with two breeding lines of NRF bulls, Aass (1996) found a heritability of 40% for tenderness. A follow up study on genetic causes of variation in beef tenderness support these results (Aass et al., 2005). As a part of this study microscopic structural changes in the muscle during storage was quantified and related to the WB tenderness in 28 randomly selected animals. This was done in order to elucidate individual variation in post mortem structural changes and to identify main structural featuresaffecting WB tenderness.

Objectives

The aim of the present study is to investigate the relative importance of structural changes in myofibers and connective tissue for variability in beef tenderness, including

studies of the post mortem glycolysis and calpastatin activity that may be related to inherent causes of tenderness in Norwegian Red Cattle.

Methodology

The 28 bulls used in this study were slaughtered and handled as described by Aass et al. (2005). Muscle tissue samples for enzyme and inhibitor activities were collected together with a hot-boned sample of the loin (M. *Logissimus dorsi*; 10^{th} thoracic to 2^{nd} lumbar vertebrae). The loin was immediately vacuum-packed and conditioned at 12 ^{0}C for 10 h before ageing at 4 ^{0}C for 7 days, followed by measurements of Warner-Bratzler shear force (WB).

For WB analyses, meat slices (3.5 cm thick) were vacuum packed, heated in a water bath at 70°C for 50 min and chilled in ice water for 45 min. Slices (1cm thick) were cut twice in the fibre direction to give samples (ten replicates) of 2x1x1cm^3 which were cut using a WB force device (triangular version) in an Instron Materials Testing Machine.

PH were determined 1, 6, 10 and 48 hours pm. The pH were measured with a Knick Kicroprocessor pH Meter Portamess 752, Electrode Mettler Toledo InLab 427

The enzyme analyses include the activity of m-calpain, μ-calpain and calpastatin, according to the procedures described by Shackelford et al. (1994) with some modifications. Only the calpastatin results will be reported here. The calpastatin assay as described by Aass et al (2005) was performed 24-31hrs pm.

Muscle samples for microscopy were taken 7 days pm from the adjacent slices to those used for WB measurements. Muscle blocks of 2 x 2 x 3 mm^3 were fixed in 2.5% glutaraldehyd in cacodylate buffer and embedded in plastic resin as previously described by (Ofstad et al., 1993). For all samples, 3μm thick plastic sections were cut both perpendicular and longitudinal to the fibres. Optical microscopy observations were of sections stained in 0.1 g/100 ml toluidine blue dissolved in 0,1Msodium acetate solution. At the light microscopic level the evident changes included fibre contraction, fibre detachment, and partial and full break in the fibres. Optical microscopy with a 20x objective was used to measure myofibre to myofibre detachment and myofibre to perimysium detachment. Quantification of structural changes was performed as previously described by Taylor and Frylinck (2003). This involved counting the numbers of myofibres attached and partly or completely detached from the neighbouring myofibre for a minimum of 200 myofibre-to-myofibre attachments on transversally sectioned myofibres. In addition, myofibre-to-perimysium detachments were 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. Contracted and broken fibres, partly and totally, were counted on a minimum of 30 fibres on longitudinally sectioned muscle fibres.

In order to estimate correlation between design variables (X) myofibre-myofibre (F-Fd), myofibre-perimysium (F-Pd) detachments, pH, calpastatin and theY-variable tenderness (WB) we used partial least squares regression (PLSR). The data analysis was performed by using Unscrambler® 9.1 (Camo AS, Oslo, Norway, 2004).

Results & Discussion

The WB values for the 28 bulls are presented in Figure 1. The values represent typical variation in tenderness for LD of NFR bulls. Genetic factors account for a significant part of the variation observed in WB tenderness (Aass et al., 2005).

The light micrographs in Figure 2 illustratestructural changes in 7 days pm LD muscle with high and low WB-values (white columns). Myofibre-myofibre and myofibre-perimysium detachments are indicated with arrows in Figures 2a and c. Fibre contraction (asterisk) is evident as waves and contraction bands as shown in Figure 2b. Fully and partly broken fibres are marked with arrows in Figure 2d. The mean values of the muscle structure changes are given in Table 1. The mean values of the structural changes are in accordance of those reported for some African breeds by Taylor and Frylinck (2003). The very large standard deviations however indicate large individual variations among the 28 bulls examined in this study.

In the tough muscle somegaps can be seen between the myofibres, whereas the perimysium seems quite intact (Fig 2a). In the tender muscle loss of myofibre-myofibre attachments as well as loss of myofibre-perimysium attachments are clearly evident (Fig 2b). In average for all the samples 65% of the myofibres were detached and 38% of the myofibre were detached from the perimysium (Table 1). Previous studies have shown that this is due to costamere degradation by calpain (Taylor et al., 1995; Koohmaraie, 1996). Recently, Hannesson et al (2004) showed that in bovine LD muscle widening of the endomysial sheaths, being evident after 1 day of storage, were also caused by break down of the matrix components, i.e. large proteoglycans and hyaluronic acid. Our results indicate that the myofibre-perimysium attachments are more stable than the myofibre-myofibre attachments.

It is well established that proteolysis of myofibrillar proteins leads to increased fragmentation of myofibrils during post mortem storage (Taylor and Koohmaraie, 1998; Ho et al, 1996). However, most of the quantitative changes described have been at the ultrastructural level; i.e. fractured myofibrils. Minimum size of structures which can be sensory perceived is estimated to 100 μm (Hatae, 1990). This corresponds to the size of two fibres, and would include breaks of the whole fibres and myofibre to myofibre adhesion (Taylor and Frylinck, 2003). Figure 2d shows that in the sample with the lowest WB value the myofibres are severe fractured both partly and in the entire width of the myofibre. The though sample (Fig 2b) possessed more contracted fibres than the tender one.

Table 2 shows mean values of calpastatin and pH at 1, 6, 10 and 48 hrs pm. The pH fall over this period was app. 1 unit, and there were only minor differences in ultimate pH among the animals. The pH will thus not be included in the regression model.

Figure 3 shows the correlation plot for Factor 1 versus Factor 2 from a PLS regression with structural changes and calpastatin as design parameters (X) on WB values as Y-variables. Both, the X variables and the WB values were weighted by their standard deviations prior to PLSR. The inner and outer circles indicate 50% and 100% explained variance in the model, respectively. The first factor explains 23% of the total variance of the design variables, which models 37% of the variance in the measured WB values. Factor 2 explains 24% of the variance in the design variables modelling 7 % of the Y variance. The X-loading shows that fibre breaks govern most the variation in the first factor and is negatively correlated to the WB values. Myofibre-perimysium detachment

and calpastatin activity, being opposite correlated are the main factors explaining most of the variation in the second factor. Myofibre-myofibre detachment and fibre contraction, being opposite correlated, have less effect on the 7 days measured WB values. However, the myofibre-myofibre detachment may have an impact on the degree of fibre contraction which also influence meat quality (Herring, 1965). Previous studies have shown that the myofibre-myofibre detachments occur within 24 hrs pm (Hannesson, 2003, Taylor and Frylinck, 2003). Such processes may have an impact on the post mortem structural changesand should be further studied.

The correlation plot in Figure 3 indicates that myofibre breaks are the main structural factors responsible for the variation in WB values. Frylinck and Heinze (2003) reported that WB shear force was significantly associated with fiber breaks at day 21.. The myofibre detachment or contraction had no effect on the WB values. However, it is possible that this effect had been larger if texture had been measured on uncooked samples. In fish connective tissue degradation was associated with loss of texture hardness (Taylor et al., 2002).

The breaks in the fibres are probably due to reduced calpastatin activity, which allows higher calpain activity (Taylor and Koohmaraie, 1998). In this study, the correlation between calpastatin activity and breaks were –0.45 (22 animals). Aass et al (2005) reported that the WB was positively related to an increase in calpastatin activity (r_P = 0.44) when analysed on 98 bulls. Both WB and calpastatin activity showed some heritability, i.e. 0.20 and 0.22, respectively. This study clearly demonstrated that structural variations influence variation in meat quality, which may be due to genetic differences within a breed.

Conclusion

This study shows that there is considerable individual variation in both post mortem myofibre detachments and breaks in the myofibres in LD muscle in NFR bulls. Fibre breaks are probably the main cause for the variation in WB tenderness measured 7 days p.m. However, due to the large individual variation in connective tissue decomposition between the bulls,the importance of such processes for the meat quality can not be ignored. In general the myofibre-perimysium attachments were more stable than the myofibre attachments. In addition the preliminary results of this study imply that structural changes and WB tenderness are closely related to the calpastatin activity. In a parallel study, with a larger number of bulls, it was shown that genetic factors accounted for a significant part of the variation observed in calpastatin activity and WB tenderness.

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Table 1. Structural changes 7 days pm. Values are the mean ± standard deviation of 100 fibres of each of 28 animals.

Myofibre – myofibre detachments	Myofibre-perimysium detachments	Broken Myofibres	Contracted Myofibres
65±31	38±22	27±32	18±20

Table 2 Values are the mean ± standard deviation of 22 animals.

pH 1h	pH 6h	pH 10h	pH 48h	Calpastatin (Units/gr)
6.6± 0.2	6.1± 0.2	5.8± 0.2	5.5± 0.1	2.5±0.6

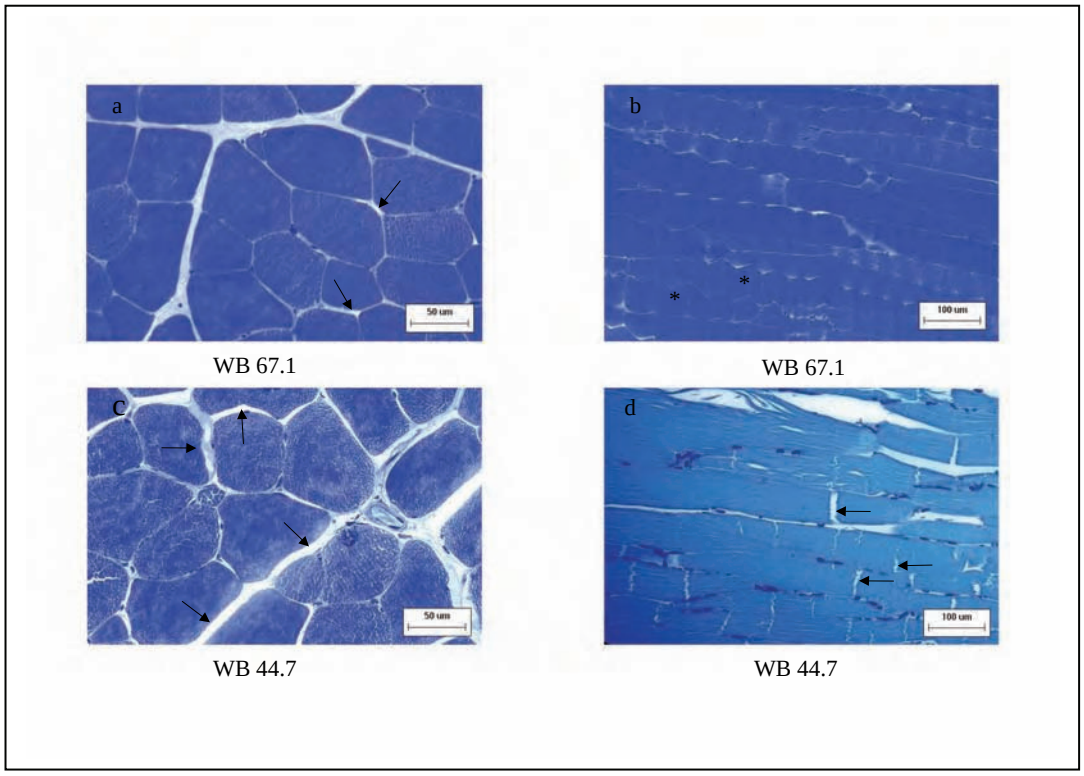

Figure 1. Light micrographs of LD muscle with high (a-b) and low (c-d) WB values at 7 days pm. Myofibre-myofibre and myofibre-perimysium detachments are indicated with arrows in (a and c). Fibre contraction (asterisk) is evident as waves and contraction bands in (b). Fully and partly broken fibres are marked with arrows in (d).

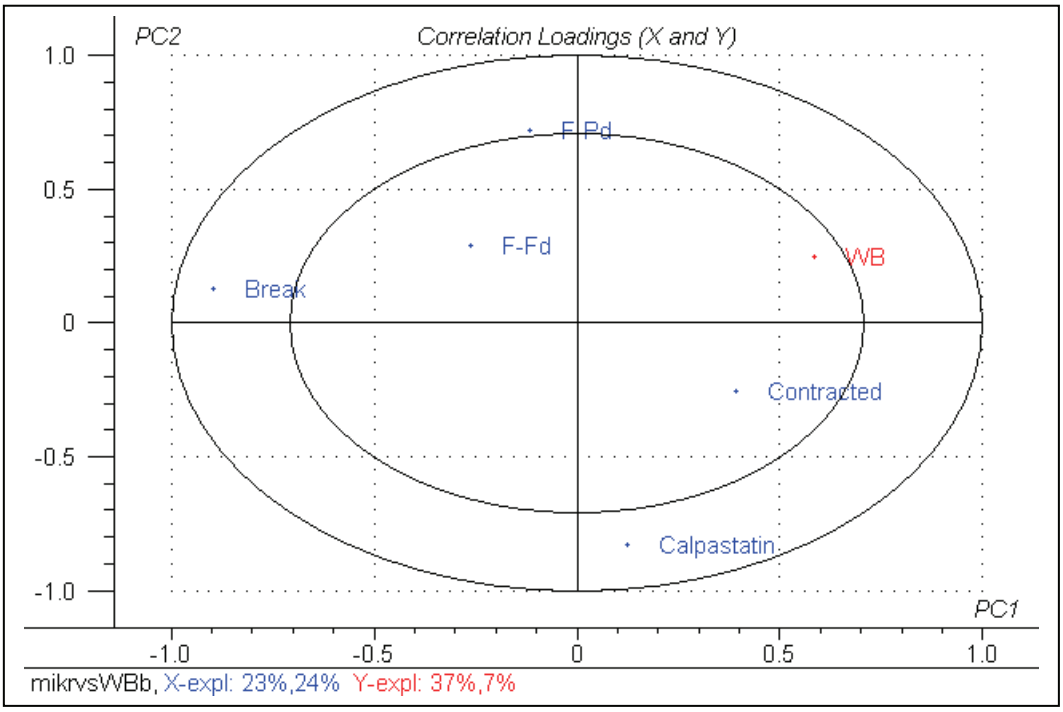

Figure 2. Correlation plot for Factor 1 versus Factor 2 from a PLS regression with structural changes and calpastatin as design parameters (X) on WB values as variables (Y).