# PROTEOLYTIC ACTIVITY AND PROTEIN CHANGES IN FOUR DIFFERENT MUSCLES WITH VARYING MEAT TENDERNESS LEVELS FROM NORWEGIAN RED HEIFERS

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**Abstract – The objective was to investigate the relationship between *postmortem* proteolytic activity, global protein changes, and meat tenderness in four different muscles (*Longissimus dorsi*, LD; *Rectus femoris*, RF; *Infraspinatus*, IS; *Vastus lateralis*, VL) from Norwegian Red heifers subjected to different pastures. Meat tenderness was measured by Warner-Bratzler shear force (WBSF), proteolytic activity was measured as calpain-specific degradation of Troponin-T (TnT) and the activity of the pro and active form of matrix metalloprotease 2 (MMP-2), and global protein changes were analyzed by 2D-DIGE proteome analysis. The IS muscle had a significantly reduced WBSF level compared to the other muscles, however it also showed very limited calpain-mediated proteolysis and a low MMP-2 activity. These results indicate that *postmortem* proteolysis seems to play a less central role for tenderness determination in the IS muscle. We also found that the LD muscle had a higher level of calpain-mediated proteolysis than all the other muscles, and that its MMP-2 activity was higher than the IS, but equal to the VL and RF muscles. In summary, the *postmortem* proteolytic activity varied between the LD, IS, VL and RF muscles, however this variation cannot explain all the differences observed in meat tenderness between these muscles.**

## I. INTRODUCTION

Tenderness is a critical factor determining the consumer's acceptance of meat, and unfortunately considerable variation in tenderness is found between different cuts and muscles. One of the main determinants of meat tenderness is calpain-mediated proteolysis of key myofibrillar and associated proteins during *postmortem* cooler storage of meat [1]. These proteins maintain the structural integrity of the myofibrils, and once degraded the rigid structure of the myofibrils is weakened leading to muscle fibre breakage and more tender meat [2]. Moreover, a possible role of matrix metalloproteases (MMPs) in the breakdown of connective tissue in meat has been suggested [3]. The expression of MMPs in muscle fibres are also found to depend on the muscle fibre type [4], and could therefore potentially play a role in explaining some of the variation in tenderness between different bovine muscles.

The objective of this study was therefore to investigate the relationship between *postmortem* proteolysis, measured as calpain-specific degradation of Troponin-T (TnT) and the activity of the pro and active form of MMP-2, general protein changes by 2D-DIGE proteome analysis, and meat tenderness (Warner-Bratzler shear force) in four different muscles; *Longissimus dorsi* (LD), *Rectus femoris* (RF), *Infraspinatus* (IS), and *Vastus lateralis* (VL), from Norwegian Red heifers subjected to fertilized conventional pasture or natural land with no fertilizer.

## II. MATERIALS AND METHODS

Twelve Norwegian Red heifers were either subjected to fertilized conventional pasture (Group 1) or natural land with no fertilizer (Group 2). Due to climatic reasons the animals had to be fed indoor from October to May. During this period all animals were given the same feeding, 1kg concentrate and roughage ad libitum. All animals were born in December and had two summers on pasture. Group 1 was slaughtered at the age of 20 months while group 2 was 23 months old at slaughter. The animals were slaughtered at a commercial abattoir, and samples were collected from four different muscles (*Longissimus dorsi*, LD; *Rectus femoris*, RF; *Infraspinatus*, IS; *Vastus lateralis*, VL) following 2 and 14 days of cooler storage. At both time points, samples were snap frozen in liquid nitrogen and stored at -80°C for determination of TnT-degradation, MMP-2 activity and 2D-DIGE analysis, while WBSF was measured on fresh samples at 14 days *postmortem*.

For Warner-Bratzler shear force (WBSF) measurements, slices of all the muscles (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. Small samples were then cut from the cooked meat with slices parallel to the fiber direction. This produced 10 samples (2x1x1cm) which were sheared using a WBSF triangular version device mounted in an Instron Materials Testing Machine. The average results from the 10 samples were used for each muscle in the statistical analysis.

Degradation of TnT was measured as the occurrence of a calpain-specific 30-kDa fragment analyzed by Western blot, while the activity of MMP-2 was measured by gelatin-zymography. From the LD muscle of all animals, muscle was also snap frozen 1h after slaughter, and water-soluble proteins from 1h and 14 days *postmortem* were analyzed by DIGE labelling and 2-dimensional gel electrophoresis. The gel images were then analyzed using the SameSpots software (version 4.5), and statistical analysis of the spot volumes were performed using the q-value calculation in SameSpots and with Partial Least Squares Regression (PLSR) with Jack-knife. Significantly altered protein spots were identified by MALDI-TOF/TOF mass spectrometry.

Analysis of variance for WBSF, TnT degradation and MMP-2 activity was performed using MINITAB's general linear model (Minitab, version 16.1.1). The models for TnT and MMP-2 included the following factors: animal group, muscle, ageing period, and all two-factor interactions. The model for WBSF included the following factors: animal group, muscle, and their interaction. The significant level was set to $p < 0.05$, and when the effect of a factor was significant, means were separated using the Tukey method.

## III. RESULTS AND DISCUSSION

Results from the WBSF measurements showed a significant interaction between animal group and muscle (Fig. 1), and the IS muscle had lower WBSF values (i.e. was more tender) compared to all other muscles for group 1, and lower values compared to the VL from group 2. There were no group-differences in WBSF within the individual muscles.

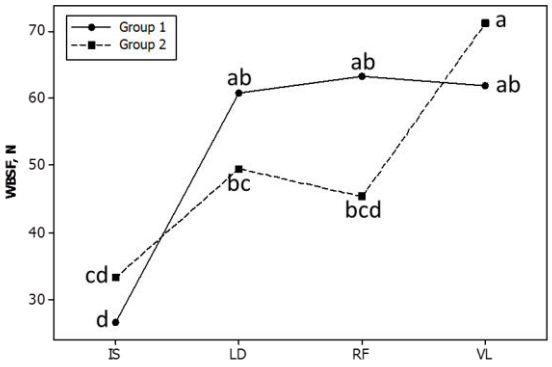


Figure 1. Warner-Bratzler shear force in four different muscles from the two animal groups measured at 14 days *postmortem*. Means with different letters are significantly different ($p<0.05$).

Occurrence of the calpain-specific 30-kDa TnT fragment did not differ between the two animal groups, however a significant interaction effect was seen between muscles and ageing period (Fig. 2). At 2 days *postmortem* the level of this fragment was very low, and no differences were seen between the different muscles. At 14 days *postmortem* however, the LD had a higher occurrence than RF and VL, while the IS showed no increase during the storage period indicating very limited calpain-mediated proteolysis.

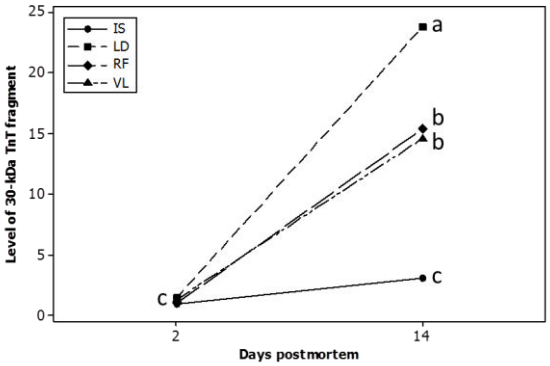


Figure 2. Occurrence of the 30-kDa Troponin-T fragment in four different muscles at 2 and 14 days *postmortem*. Means with different letters are significantly different ($p<0.05$).

Matrix metalloproteases exist in two different forms *in vivo*; an inactive pro-form (pro-MMP-2) and an active form (MMP-2). The gelatin-zymography method enables us to quantify both of these forms of this protease. For pro-MMP-2 there was a significant interaction effect between muscles and ageing period (Fig. 3). At 2 days *postmortem*, the LD muscle had a lower activity

of pro-MMP-2 compared to the RF and VL, while the IS showed no difference to any of the other muscles. The lower amount of pro-MMP-2 in the LD may indicate that this muscle has had a greater activation of this protease during this storage period. After 14 days of ageing no differences were observed between the muscles regarding this inactive pro-form of MMP-2. For the active MMP-2, there were no interaction effects, but significant main effects for animal group, muscle and ageing period. The activity was higher in animal group 1 compared to group 2, reduced at 14 days *postmortem* compared to 2 days, and the IS muscle had a significantly lower MMP-2 activity than the LD muscles, while RF and VL were not different compared to each other and the other two muscles. These results indicate that this protease is activated in all these muscles during *postmortem* storage, and that the MMP-2 activity varies between these muscle types and thus could explain some of the tenderness differences observed between them.

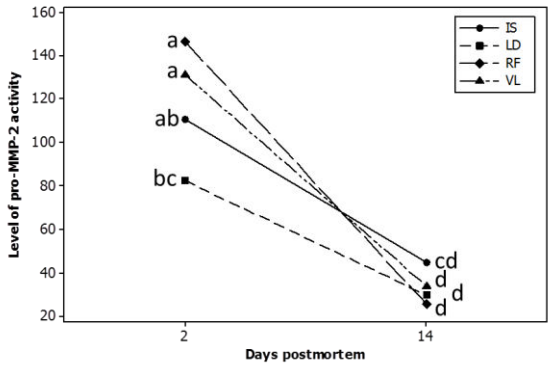


Figure 3. The pro-MMP-2 activity in four different muscles at 2 and 14 days *postmortem*. Means with different letters are significantly different ($p$<0.05).

From the 2D-DIGE analysis, no significant changes were found between the two animal groups however, 52 protein spots were significantly changed in abundance between the two time points at 5% significance level (Fig. 4). Of these 52 protein spots, 16 were successfully identified by MALDI-TOF/TOF mass spectrometry (Table 1). In the samples collected at 1h *postmortem* we observed an elevated abundance of small heat shock proteins, enzymes involved in glycolysis and energy metabolism and myosin light chain as compared to the 14 day samples. At 14 days *postmortem* heat shock protein 70, serum albumins, phosphoglucomutase-1 and adenylate kinase had elevated abundances. These changes agree with several other proteome studies of *postmortem* changes in muscle, showing increased energy metabolism and oxidative stress conditions during the very early *postmortem* stages [5].

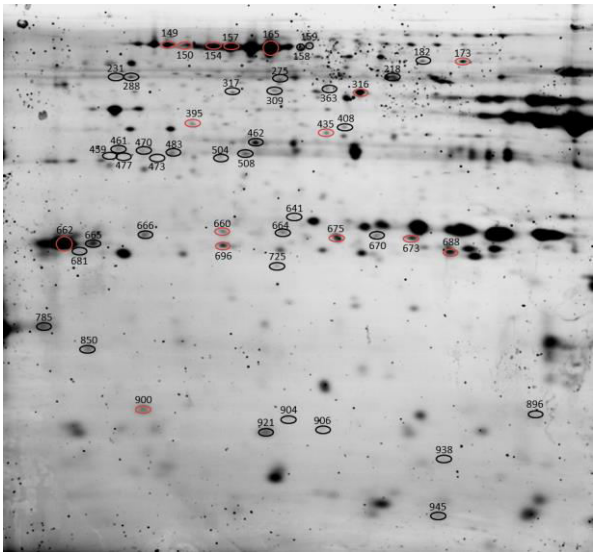

Figure 4. Representative 2-DE gel image (pI 5-8, 12% acrylamide). Proteins altered in abundance between 1h and 14 days *postmortem* are marked.

Table 1 Proteins showing significant alternation in abundance from 1h to 14d *postmortem* in the *Longissimus dorsi* muscle

| Spot no | Protein name (source: *bos taurus*) | Ratio 1 h / 14 d |
|---|---|---|
| 149 | Heat shock protein 70 | 0.1 |
| 150 | Stress-70 protein | 0.6 |
| 154 | Serum albumin | 0.7 |
| 157 | Serum albumin | 0.7 |
| 165 | Serum albumin | 0.7 |
| 173 | Phosphoglucomutase-1 | 0.6 |
| 316 | Alpha-enolase | 1.9 |
| 395 | Ankyrin-repeat domain protein | 5.7 |
| 435 | Isocitrat dehydrogenase | 2.5 |
| 660 | Heat shock protein beta-1 | 4.6 |
| 662 | Fast-twich myosin light chain | 9.3 |
| 673 | Heat shock protein beta-1 | 12.0 |
| 675 | Heat shock protein beta-1 | 15.0 |
| 688 | Adenylate kinase isoenzyme 1 | 0.2 |
| 696 | Myosin light chain | 7.7 |
| 900 | Phosphohistidine phosphatase | 2.8 |

Thus, there was a considerable variation in tenderness between the four muscles in this study, with the IS muscle having about 50% reduced WBSF values compared to the other muscles. However the IS muscle also had the lowest occurrence of the 30-kDa fragment of TnT, indicating very limited calpain-mediated proteolysis. Similarly, the IS muscle also had a

reduced MMP-2 activity compared to the LD muscle. These results indicate that *postmortem* proteolysis seems to play a less central role for the tenderness determination in this muscle. Moreover, the results show that the LD muscle has a higher level of calpain-mediated proteolysis than the other muscles, and that the MMP-2 activity in this muscle was higher than in the IS, but equal to the VL and RF muscles.

## IV. CONCLUSION

The *postmortem* proteolytic activity varied between the LD, IS, VL and RF muscles, however this variation cannot explain all the differences in meat tenderness observed between these muscles. Information on the *postmortem* tenderization potential for different beef muscles provides the meat industry with useful knowledge regarding optimal handling and storage procedures for different beef muscles.

## ACKNOWLEDGEMENTS

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