THE EFFECT OF ELECTRICAL STIMULATION ON *POST MORTEM* MYOFIBRILLAR PROTEIN DEGRADATION AND αβ-CRYSTALLIN KINETICS IN BULL BEEF

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Abstract – The aim of this paper was to determine the effect of electrical stimulation on shear force, myofibrillar protein degradation and αβ-crystallin kinetics in bull beef during ageing. *M. longissimus lumborum* from either the left or right side of bull carcasses (n = 15) were low-voltage electrically stimulated (ES), with the other side not stimulated (NS). Muscles were categorized into low (pH < 5.8), intermediate (pH 5.8 – 6.19) and high (pH ≥ 6.2) ultimate pH (pH_u) and held at 15°C until 24 h *post mortem* then aged at -1.5°C for up to 28 d *post mortem*. Titin and desmin degradation and αβ-crystallin concentrations were measured at 0.5 h, 3 h, 6 h, 12 h, 18 h, 1 d, 2 d, 7 d, 14 d and 28 d *post mortem*. Shear force was also determined for all samples at 1, 2, 7, 14 and 28 d *post mortem*. High pH_u meat attained acceptable tenderness at earlier aging time and was significantly more tender than the other pH_u groups at all ageing timepoints ($p < 0.05$). Titin and desmin degradation also occurred earlier in high pH_u meat. Electrical stimulation did not significantly affect shear force or the degradation rates of titin and desmin. However, αβ-crystallin concentration in ES muscles was significantly higher at latter *post mortem* ageing timepoints ($p < 0.05$).

Key Words – tenderness, small heat shock proteins, ultimate pH

I. INTRODUCTION

Inconsistent tenderness is an ongoing issue for the meat industry. Tenderization of meat is widely attributed to the degradation of myofibrillar proteins by endogenous proteolytic enzymes during *post mortem* storage. Furthermore, it is known that the rate and extent of pH decline influences muscle protein degradation as well as proteolytic enzyme functions, thus affecting meat tenderness. A recent study reported that high pH_u bull beef was already acceptably tender at 1 day *post mortem* and significantly more tender than low pH_u beef [1]. However, the mechanisms driving meat tenderization on the basis of initial pH decline and pH_u during *post mortem* glycolysis are unclear.

Small heat shock proteins (sHSPs) such as αβ-crystallin are involved in preventing irreversible protein aggregation and damage during stress [2]. They are also involved in the regulation of apoptosis, a type of programmed cell death proposed to be important to meat quality [3]. As sHSP have been found to be associated with myofibrillar proteins and the assembly of muscle filaments [4], they have been implicated in meat tenderization.

Low voltage electrical stimulation of beef carcasses is commonly practiced in New Zealand. This is done by running a current through whole carcass following carcass immobilization. The effects of electrical stimulation on the myofibrillar protein degradation and αβ-crystallin kinetics in muscle during *post mortem* ageing and the consequences of this on meat tenderness is investigated in this study.

II. MATERIALS AND METHODS

A. Animals and sampling

Bulls (n = 15) used in this study were electrically stunned and the carcasses immobilized following exsanguination. The *M. longissimus lumborum* (LL) from both sides of the carcass were hot-boned within 30 minutes *post mortem*. Low-voltage electrical stimulation (peak voltage = 104 V) was applied to the LL muscle from one side of the carcass for 30 seconds (ES). The LL from the opposite side of the carcass was not stimulated (NS). About 10 g was excised from all muscle samples, frozen in liquid nitrogen and stored at -80°C until further analysed (0.5 h). All muscles were then packed separately into vacuum

bags then stored at 15°C until 24 h *post mortem*. All muscles were subsequently aged at -1.5°C for up to 28 d *post mortem*. About 10 g muscle samples were excised from all muscles at 3 h, 6 h, 12 h, 18 h, 1 d, 2 d, 7 d, 14 d and 28 d *post mortem* at random locations along the muscle, frozen in liquid nitrogen and stored at -80°C until further analysed.

B. pH and shear force measurements

The pH of all samples at 0, 3, 6, 12 and 18 h *post mortem* was measured using the Iodoacetate Method. The pH of samples at 1, 2, 7, 14 and 28 d *post mortem* was measured with a Testo 230 pH meter (Lenzkirch, Germany). The shear force of all samples at 1, 2, 7, 14 and 28 days *post mortem* was measured with a MIRINZ tenderometer as described by Pulford *et al.* [5].

C. Extraction of bovine myofibrils and protein measurement

Myofibrillar extracts were prepared for all animals at all timepoints as described by Wan *et al.* [6] with minor modifications. The protein content of all extracts was determined using the Biuret Method [7].

D. Coomassie blue SDS-PAGE and Western blot analysis

Titin and desmin from whole muscle extracts were resolved on 5% and 12.5% pre-cast Tris-HCl gels (BioRad), respectively. Electrophoresis was conducted in a BioRad Criterion cell system. Following electrophoresis, the gels were stained with Colloidal Coomassie blue solution for 48 h and the gel image captured with a GS700 densitometer scanner (BioRad). Western blot analysis for desmin was conducted as described by Pulford *et al.* [5].

E. Quantitative determination of αβ-crystallin

αβ-Crystallin was measured by indirect enzyme-linked immunsorbent assay (ELISA) as described by Pulford *et al.* [2] and Lomiwes *et al.* [8] with minor modifications. Myofibrillar extracts were initially diluted to 4 µg mL^{-1} with coating buffer.

F. Statistical analysis

The model time*stimulated/non stimulated + (Animal+ *L.lumborum*)/Time was fitted to pH data. The variables αβ-crystallin was analysed on the log scale to make the error variance more constant.

III. RESULTS AND DISCUSSION

High pH_u meat samples were significantly more tender than the other pH_u groups at all timepoints ($p < 0.05$). There was no significant difference in shear force between low and intermediate pH_u meat at all timepoints ($p > 0.05$). With 11 kgF as the upper limit for acceptable meat tenderness, high pH_u meat was already acceptably tender at 1 d *post mortem*. Intermediate and low pH_u meat did not attain acceptable tenderness until about 7 d *post mortem*. No significant difference in shear force was observed between NS and ES muscles for all pH_u groups ($p < 0.05$).

Titin degradation product, Titin 2 (T2), was initially detected in high and intermediate pH_u meat at 0.5 h *post mortem* (Figure 1). However, titin degradation occurred faster in high pH_u meat followed by intermediate then low pH_u meat. Titin degradation was not detected in low pH_u meat until 7 d *post mortem*. Similarly, desmin degradation occurred earlier and faster in high pH_u meat, as indicated by the b1 and b2 bands at 3 h and 12 h, respectively (Figure 2). The rate and extent of desmin degradation in low and intermediate pH_u meat were similar. No apparent difference to the extent and rate of titin and desmin degradation was observed between representative NS and ES muscles for all pH_u groups.

Myofibrillar αβ-crystallin concentrations progressively increased after slaughter and peaked at approximately 2 days *post mortem*, then plateau at subsequent ageing timepoints (Figure 3). Although αβ-crystallin concentration was not influenced by pH_u, the expression of αβ-crystallin was affected by electrical stimulation. αβ-crystallin concentrations were significantly lower early *post mortem* in ES samples, then dramatically increased between 1 d and 2 d *post mortem* so that at 2 d *post mortem* and proceedings timepoints, αβ-crystallin concentrations in ES muscles were significantly higher compared with NS muscles for all pH_u groups.

Based on the results of this study, pH_u had a more significant effect on shear force and the degradation of myofibrillar proteins compared with electrical stimulation.

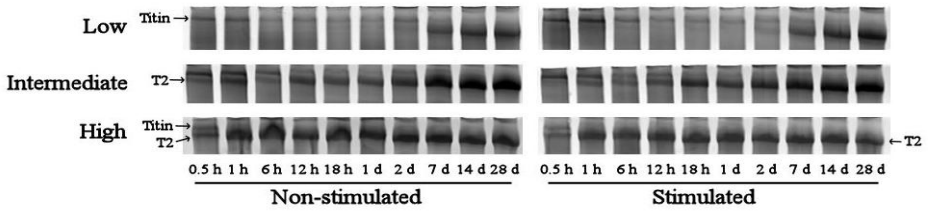

Figure 1: Degradation of titin in representative low, intermediate and high pH_u NS and ES bull beef during *post mortem* ageing.

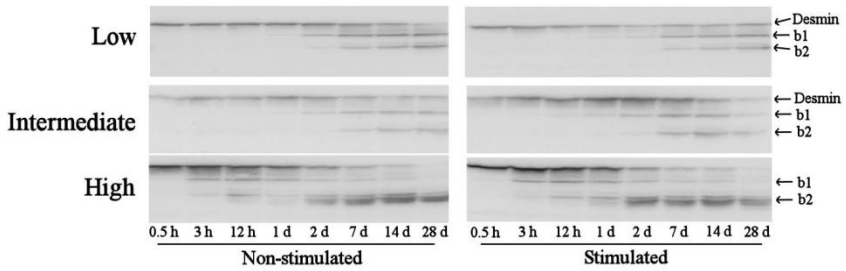

Figure 2: Degradation of desmin in representative low, intermediate and high pH_u NS and ES bull beef during *post mortem* ageing.

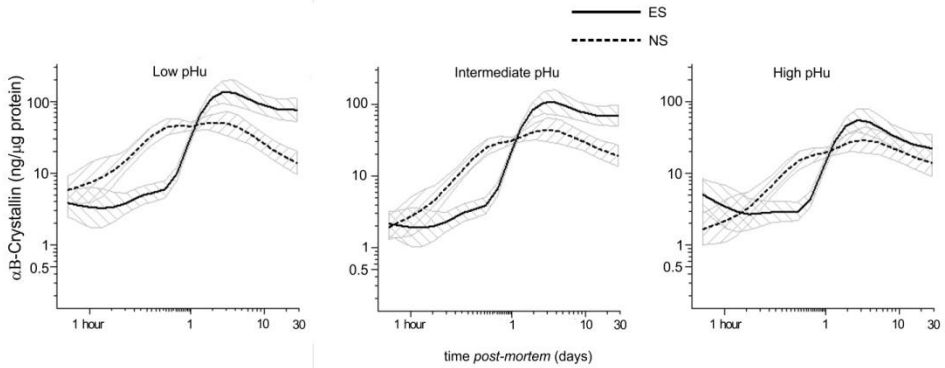

Figure 4: αβ-Crystallin concentrations in NS and ES muscles from each pH_u category during *post mortem* ageing

The faster degradation of intact titin in high pH_u meat was concomitant with the rapid tenderization of this pH_u group. Inversely, slower attainment of acceptable tenderness in low and intermediate pH_u meat is concurrent with the slower degradation of intact titin. Thus tenderization in these groups is attributed to the combined degradation of large and small molecular weight proteins such as titin and desmin, respectively, during ageing.

Electrical stimulation was found to increase the tenderness of meat [9]. It is important to consider that in this study, both NS and ES samples were subjected to electrical stunning and immobilisation. It is proposed that the effect of electrical stimulation is virtually nullified as muscles have already received sufficient electrical inputs through these mandatory industry practices and may explain the equivalent shear force and myofibrillar degradation profiles between the two treatments.

The decline of αβ-crystallin in muscle sarcoplasm particularly in low pH_u meat was attributed to protein precipitation as muscle pH declined beyond the protein's isoelectric point during ageing [2 and 10]. With the increase of αβ-crystallin in the myofibrillar fraction, as reported in this study, it is proposed that αβ-crystallin translocates from the sarcoplasm to the myofibrillar phase of muscle *post mortem* [2]. Greater levels of αβ-crystallin in ES meat at latter

ageing timepoints may be attributed to stress induced by directly electrically stimulating the muscle. However, there was no significant difference in the shear force between NS and ES muscles suggesting that αβ-crystallin at the level induced by ES may not have a significant effect on the tenderization rate of beef.

It is important to reiterate that the shear force of intermediate and low pH_u meat were equivalent throughout ageing. This explains the similar degradation profiles of titin and desmin and αβ-crystallin kinetics between these two groups. Intermediate pH_u meat is widely known for being inconsistently tender. However, none of the intermediate pH_u muscles collected in this study yielded tough meat. Thus the effect of electrical stimulation on myofibrillar protein degradation and small heat shock protein kinetics, particularly in tough intermediate pH_u meat, requires further investigation.

IV. CONCLUSION

The results of this study found that the direct application of low-voltage electrical stimulation on muscle did not significantly affect the rate of meat tenderization or myofibrillar protein degradation in any of the pH_u groups. In contrast, ES muscles had significantly higher levels of αβ-crystallin after 1 d *post mortem* in all pH_u groups. However, greater levels of αβ-crystallin in ES muscles did not affect meat tenderness suggesting that the level of sHSPs induced with ES may not be sufficient to affect the tenderization of pH_u beef. As none of the intermediate pH_u muscles in this study yielded unacceptably tough meat, further work to determine the effect of electrical stimulation on shear force, myofibrillar protein degradation and small heat shock protein kinetics on tough intermediate pH_u meat is proposed.

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