# ULTRASTRUCTURAL CHARACTERISTICS OF BEEF LOIN MUSCLE TREATED WITH HIGH OR LOW VOLTAGE ELECTRICAL STIMULATION

I.H. Hwang, and J.M. Thompson,
Co-operative Research Centre for the Cattle and Beef Industries, University of New England, NSW, 2351, Australia.

**Background**

A number of workers have suggested that physical disruption of the myofibrillar complex is one of the mechanisms whereby electrical stimulation improves meat tenderness. The formation of contracture bands, which contain areas of stretched, ill-defined or disrupted sarcomeres, and increased proteolysis, are identified as the most convincing evidence (Will *et al.*, 1980; Sorinmade *et al.*, 1982). Ho *et al.* (1996) reported that both contracture nodes and proteolysis contribute to improvements in tenderness. They subjectively counted the number of fractures and reported significantly higher fracturing of the I-band adjacent to the Z-line and increased degradation of titin, nubulin, desmin and tropoin-T following high voltage electrical stimulation of bovine muscle. In contrast, McKeith *et al.* (1980) showed that although high voltage stimulation of intact and half carcass resulted in differences in ultrastructural damage, there were no differences in peak force measurements. Fabiansson and Libelius (1985) found contracture nodes in both stimulated (low voltage) and non-stimulated carcasses and concluded that they were most likely an artifact of improperly prepared tissue preparations.

Most studies have reported an increase in proteolysis of myofibrillar proteins during ageing (e.g. Ho *et al.* 1997). However the changes in the muscle ultrastructure have generally been descriptive with few attempts to quantify the effects of the different stimulation systems, or the changes that occur with ageing.

**Objective**

The purpose of this study was to quantify the effects on muscle ultrastructure and meat tenderness of high and low voltage electrical stimulation in combination with two different cooling regimes.

**Methods**

*Animals and Experimental Design:* Animals (4 Angus x Hereford grass-fed steers) were slaughtered in a commercial abattoir. The left side of two animals were subjected to high voltage stimulation within 45 minutes post-mortem for 55 seconds, whilst the corresponding right sides served as non-stimulated controls. The output of high voltage unit was 800 volts RMS with continuous alternating polarity of bi-directional half-sinusoidal pulses with a width of 10 msec (14.3 pulses per second) and 4.3 amps. These sides were cooled at 0-2°C (air speed; 0.5 m/s). The other 2 animals were subjected to low voltage stimulation for 45 seconds, within approximately 5 minutes post mortem. The output of the low voltage unit was 45 peak volts with the alternating polarity of square wave pulses with a width of 25ms pulses with 100 ms on and 12 ms off (36 pulse per second). The left sides of low voltage carcasses were cooled at 0-2 °C and the right sides cooled at an elevated temperature (5-7 °C, air speed; 0.2 m/s).

*Measurements:* Striploins were removed 24 hours after chilling. Steaks (three steaks x 20 mm) for histological observation and muscle samples (250 g) for objective measurements were removed from M. *longissimus thoracis et lumborum* (LD) caudal from the $11^{th}/12^{th}$ rib junction, vacuum packed and aged for 1, 14 and 28 days at 1°C. Samples for the electron microscope were collected the fresh steak, whilst the objective samples were frozen at -20°C after ageing. Objective samples were thawed for 48 hours and cooked at 70°C for 60 minutes in a water bath, prior to cooling in running water for 30 minutes and chilling overnight. The cooked samples were prepared for shear force and compress measurements according to Bouton *et al.* (1971).

For the transmission electron microscope examination, samples were initially fixed with the Karnovsky's fixative followed by secondary fixation using 1 % $OsO_4$ (Karnovsky, 1965). Samples were then dehydrated in ethanol and infiltrated with Spurrs's resin (ERL 4206). Thin sections of approximately 50 µm thickness were cut and first stained in 4 % Uranyl acetate followed by lead citrate. Sections were examined with a JEOL JEM-1200 EX TEM at an accelerating voltage of 60 kV.

Microscopic images were digitized using AverMedia PC program (AverMedia Tech.), and quantitatively analyzed using an image analysis program (Leica Q500, Cambridge). Ten representative images for each sample were used. Each image comprised of approximately 10 countable sarcomeres from 6-8 myofibrils for the longitudinal section. In the case of transverse section, each image contained approximately 35-40 myofibrils. The gaps for longitudinal and transverse sections were expressed as percentages of whole image. Gaps of inter and intra myofibrils comprised white spaces between myofibrils and inter myofibrils. Sarcomere length was averaged over 100 measurements for each sample. The frequency of contracture nodes was based on counts from 15 blocks for each treatment.

**Results and discussions**

High and low voltage electrical stimulation with two cooling regimes did not result in visual differences in myofibrillar ultrastructure. Contracture nodes comprised on average 4-8 sarcomeres, with a stretched I-band often with some tearing along the side of contracture node. The form of the contracture nodes between treatments (Photograph 1) and frequency of contracture node did not appear to be related to the peak force (Figure 1), which was consistent with the results of McKeith *et al.* (1980). There was no apparent difference in proteolysis between treatments over ageing periods. Integrity of Z-line and myofibrils appeared to be disappearing at Day 1. Noticeable evidence of proteolysis was shown by degradation of the Z-line region, partly at the junction of the A- and I-band regions and by lateral separation of the myofibrils (Photograph 2).

Image analysis showed that low voltage electrical stimulation had a slightly higher percentage of gaps for the transverse section and slightly longer sarcomere length, compared with high voltage electrical stimulation and the control. However, these changes did not appear to affect meat tenderness. Comparison between high voltage electrical stimulation and control sides showed little difference between the percentage of gaps and sarcomere length (Figure 2 and Figure 3).

Even though there was only a limited number of animals examined there were some clear changes in the myofibre ultrastructure due to ageing (Figure 3). As ageing increased from 1 to 28 days the percentage of gaps in the longitudinal section increased (10.3, 16.1 and 17.5% at 1, 14 and 28 days aged, respectively). There was a similar trend for an increase in the gaps of the transverse section (14.6, 16.7 and 17.7% gaps for 1, 14 and 28 days aged, respectively), although the change between 1 and 14 days did not appear to be as great as for the longitudinal section. There was no change in sarcomere length with ageing (Figure 3). These results suggest that degradation during the initial ageing period was more evident in the longitudinal than the transverse plane. This would be consistent with degradation of Z-line region and the junction of A- and I-band during early ageing, as described by Ho *et al.* (1997). However, this does not concur with the results of Taylor *et al.* (1995), who reported earlier degradation of the costamere and intermediate filament proteins compared with the Z-line regions for non-stimulated bovine muscle.

**Conclusions**

Both high and low voltage stimulation of carcasses caused an increase in contracture nodes, but these did not appear to be related to meat tenderness. In addition, ageing seemed to be associated with degradation of Z-disk and at the junctions of the A- and I-bands.

**Pertinent literature**


Bouton, P.E., Harris, P.V. and Shorthose, W.R. 1971. *J. Fd Sc.* 36: 435-439.
Fabiansson, S. and Libelius, R. 1985. *J. Fd Sc.* 50: 39-44.
Ho, C.Y., Stromer, M.H. and Robson, R.M. 1996. *J. Anim. Sc.* 74: 1563-1575.
Ho, C.Y., Stromer, M.H., Rouse, G. and Robson, R.M. 1997. *J. Anim. Sc.* 75: 366-376.
Karnovsky, M.J. 1965. *J. of Cell Bio.* 27: 137A-138B.
McKeith, F.K., Smith, C.G., Dutson, T.R., Savell, J.W., Hosteler, R.L. and Carpenter, Z.L. 1980. *J. Fd Protec.* 43(10): 795-798.
Sorinmade, S.O., Cross, H.R., One, K. and Wergin, W.P., 1982. *Meat Sc.* 6: 71-77
Taylor, R.G., Geesink, G.H., Thompson, V.F., Koohmariaie, M. and Goll, D.E. 1995. *J. Anim. Sc.* 73: 1315-1367.
Will, P.A., Ownby, C.L. and Henrickson, R.L. ,1980. *J. Fd Sc.* 45: 21-34.

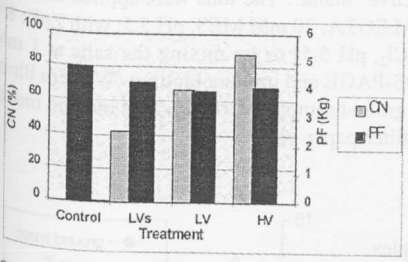




Figure 1. Comparison between the frequency of contracture node (CN) and WB-peak force (PF) for high voltage (HV), low voltage (LV), low voltage with slow cooling rage (LVs) and control. Meaned for 1, 14, 28 day ageing.

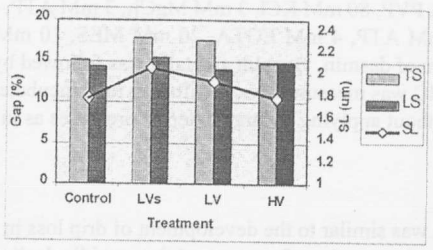



Figure 2. The gaps of transverse section (TS) and longitudinal section (LS) and of sarcomere length (SL) for high voltage (HV), low voltage (LV), low voltage with slow cooling rate (LVs) and control. Meaned for 1, 14, 28 day ageing.

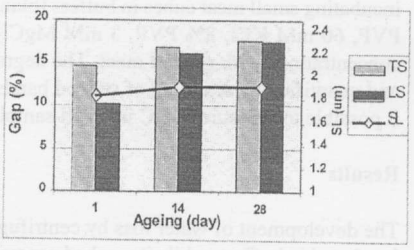
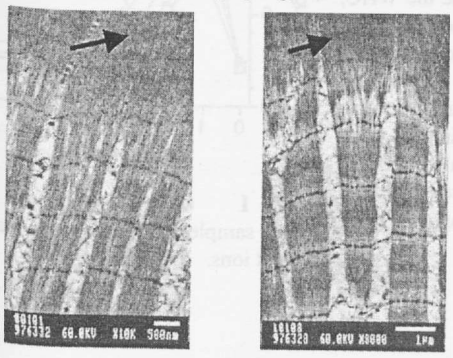
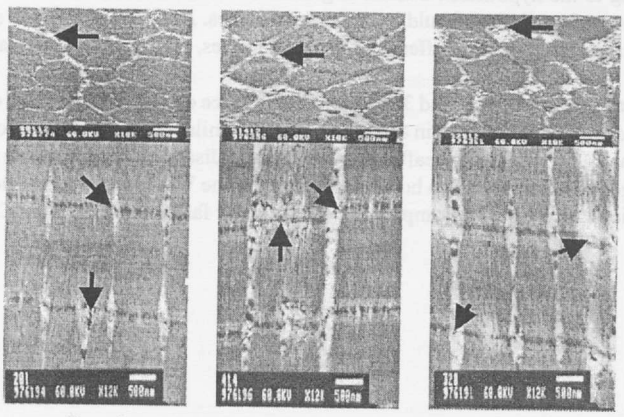



Figure 3. Changes in the gaps of transverse section (TS) and longitudinal section (LS) and of sarcomere length (SL) over ageing periods.



HV LV

Photograph 1. Representative contracture node (arrows) of high voltage (HV) and low voltage (LV) stimulation at day 1. HV: x 10 k, white bar = 0.5 μm. LV: x 8 k, white bar = 1 μm.



Day 1 Day 14 Day 28

Photograph 2. Representative ultrastructure of transverse and longitudinal section and their gaps (arrows) at ageing periods. Transverse section: x 10 K, white bar = 0.5 μm. Longitudinal section: x 12,000, white bar = 0.5 μm.