A Technique to Quantify the Extent of Postmortem Degradation of Meat Ultrastructure

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ABSTRACT : This study investigated quantitative changes in the spaces between and within myofibrils and the impact of high and low voltage electrical stimulation on muscle ultrastructure as seen in electron micrographs. In addition, the relationships of these spaces and the impact to meat tenderness were investigated. The degradation of myofibrils during aging appeared to be localized across the muscle fibre. Structural deterioration of muscle fibres was evident 1 day post-mortem, involving the weakening in the lateral integrity of the myofibrils and Z-disc regions. Meat tenderisation, as shown by objective measurements, coincided with these increases in degradation, as assessed by the sum of the gaps between and within myofibrils. The results showed that the total size of gaps between and within myofibrils can be used as an indicator of meat tenderization during aging, but that ultrastructural alteration in electrically stimulated muscle had little relationship with meat tenderness. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 1 : 111-116)***

Key Words : Beef, Aging, Tenderness, Ultrastructure, Electrical Stimulation

INTRODUCTION

A number of studies have reported changes in the muscle ultrastructure with aging, including a weakening of Z-discs, the junction of A- and I-bands or/and other integral alignments (Olson et al., 1976; Wheeler et al., 1990; Taylor et al., 1995a; O'Halloran et al., 1997). In addition to changes within the sarcomere, Robson et al. (1991) reported that much of the tenderization process included degradation of the proteins involved in both linkages between the myofibrils and in the costameres between the myofibrils and the sarcolemma. There have been some attempts to quantify the fragmentation of myofibrils (Ho et al., 1996; Taylor and Koohmaraie, 1998). Since meat tenderization is linked to ultrastructural changes not only within myofibril, but also between myofibrils, a technique that quantifies changes in gaps both within and between myofibrils with aging, or other post-mortem treatments, would be of great value.

This study investigated the quantitative changes in gaps between and within myofibrils during aging and the ultrastructural impact of high and low voltage electrical stimulation and their relationship to meat tenderness of beef *m. longissimus lumborum*.

MATERIALS AND METHODS

Animals and stimulation treatments

Four pasture finished crossbreed steers (Angus × Hereford), approximately 27 months of age, with a mean live weight of 460±56 kg, were slaughtered and the carcasses dressed using conventional methods. A downward hide puller, which incorporated the use of a rigidity probe (120 to 160 volts for approximately 15 s), was applied to all carcasses during the de-hiding procedure.

Two bodies were stimulated using a low voltage system (45 peak volts, for 45 seconds applied within approximately 5 min of death) with the left sides chilled in a slow chiller (5°-7°C, air speed 0.2 m/s, LVs) and the corresponding right sides in a conventional chiller (0°-2°C, air speed 0.5 m/s, LV). The output of the low voltage unit was 0.7 amps of 36 pulses per second (with an alternating polarity of square wave pulses with a width of 25 milliseconds with pulses 100 milliseconds on and 12 milliseconds off).

Left sides from the other two bodies were stimulated using a high voltage system (HV), with the right sides used as controls (Cont), before placing both sides in a conventional chiller. High voltage stimulation (800 volts RMS, for 55 seconds) was applied to the left sides of two carcasses, within 45 min of death using clip electrodes to the neck region and near the Achilles tendon. The output of the high voltage unit was 4.3 amps of 14.3 pulses per second (with a continuous alternating polarity of bi-directional half-sinusoidal pulses with a width of 10 milliseconds).

Sampling and measurements

pH and temperature of the caudal end of *m. longissimus lumborum* (LL) were measured at 3 and 24 h post-mortem using a portable needle-tipped combination electrode (ORION®, USA). Carcass sides were quartered at the 11th/12th rib junction and the caudal section of the LL removed at 24 h post-mortem. The LL was cut into three

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portions, vacuum packed and, within each LL, randomly assigned to one of three aging periods (1, 14 or 28 days) for objective measurements of meat tenderness. Portions were aged at 1°C, prior to freezing at -20°C until analysis. Warner-Bratzler shear force and Instron compression were measured according to the methods described by Bouton et al. (1971) and Bouton and Harris (1972).

For histological assessment, steaks (20 mm thick) were taken from the caudal end of striploin sections aged for 1, 14 or 28 days at 1°C. Karnovsky's fixative, which comprised 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 5.8 (Karnovsky, 1965), was flooded on the surface of muscle for 30 min prior to dissecting muscle bundles down to a size of approximately 4×2×2 mm. The specimens were fixed in the same fixative for a further 3 h, and then in 1% osmium tetroxide for 1.5 h at 2°C. Dehydrated specimens using a graded series of ethanol were embedded in Spurs's resin (ERL 4206, P&S Probing and Structure, Australia). Five blocks from each sample were sectioned and mounted on 200 mesh grid. Sections were positively stained in 4% uranyl acetate and 0.25% lead citrate according to Dykstra (1993). The stained sections were examined using a transmission electron microscope (JEOL JEM-1200 EX TEM, Japan) at an accelerating voltage of 60KV.

Microscopic images were digitized using the Aver Media computer program (Aver Media Tech., Taiwan), and quantitatively analyzed for changes in gaps between and within myofibrils and in sarcomere length using an image analysis program (LEICA Q500MC, Leica Ltd, Cambridge, UK). Whilst avoiding regions containing obvious artifacts, approximately 40 images were captured from each sample. Each image (×12,000) comprised approximately 10 to 12 countable sarcomeres from each of 6 to 8 myofibrils. Twenty representative images were selected for each sample for each aging time. The sum of spaces between and within myofibrils was measured and expressed as percentages of the whole image. Figure 1 shows an example of this measurement. The percentage of specimen blocks that showed contracture bands was based on assessment of five blocks from each side and aging period (i.e. a total of 120 blocks). Sarcomere length measurements were calculated as the mean of 400 individual sarcomeres measured for each treatment, for each aging period using the LEICA Q5000MC. Because of the small number of animals examined, only least square means and standard deviations on the basis of treatments within animal are presented.

RESULTS AND DISCUSSION

Ultrastructural changes during aging and their relationship with tenderness

Representative electron microscope images of normal muscle fibres and their changes during aging periods are shown in figure 2. Some disintegration of Z-disc regions, lateral attachments between myofibrils and of the attachment of myofibrils to the sarcolemma was evident at day 1. The Z-discs showed some discontinuous areas and the architecture of the sarcolemma had deteriorated. These ultrastructural changes in the muscle fibres were consistent with the results of several previous studies, which showed a reduction in the density of the Z-disc, disappearance of other integral alignments (Olson et al., 1976; O'Halloran et al., 1997) and weakening of the junction of A- and I-bands (Wheeler et al., 1990). It was noticeable that the degradation of myofibrils took place in localized areas throughout the myofibres (figure 3). This observation was not explainable on the basis of the distribution of calpains and calcium ions, since these are distributed evenly across the muscle fibre (Taylor et al., 1995b; Goll et al., 1997), although it was consistent with other electron microscope studies (Abbott et al., 1977; Ho et al., 1997). As aging time increased, there was noticeable evidence of the degradation of the Z-disc region, especially near the junction of the A- and I-bands. In addition, there was some lateral separation of the myofibrils and disconnection and disappearance of the sarcolemma.

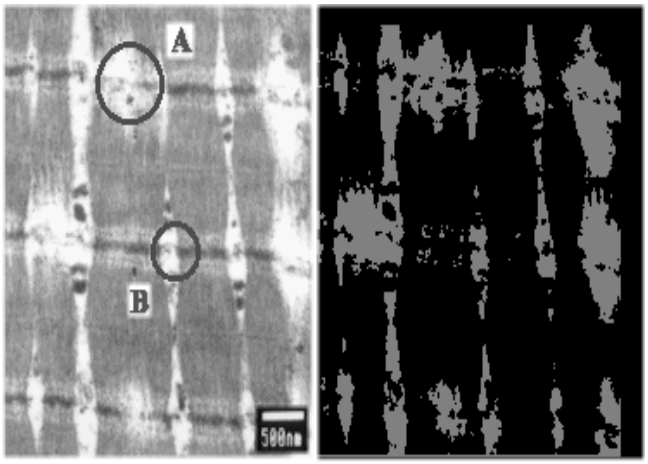

Figure 1. Representative original (1) and computer-manipulated (2) electron microscopic images, showing the measurement of gaps. In image 1, intra-myofibrillar (A) and inter-myofibrils gaps (B) are shown

As described in the methodology section, we determined the percentage of gaps between and within myofibrils as a proportion of the total area examined in longitudinal section per field (table 1). As aging time increased from 1 to 14 to 28 days, gaps increased from 10.3 to 16.1 to 17.5% of the image respectively, with most of the change occurring between 1 to 14 days post-mortem. Overall the proportion of gaps had a significant correlation with shear force ($p<0.05$; $r= -0.47$). Although a limited number of animals were involved, the results suggest that using image analysis to quantify the proportion of gaps in

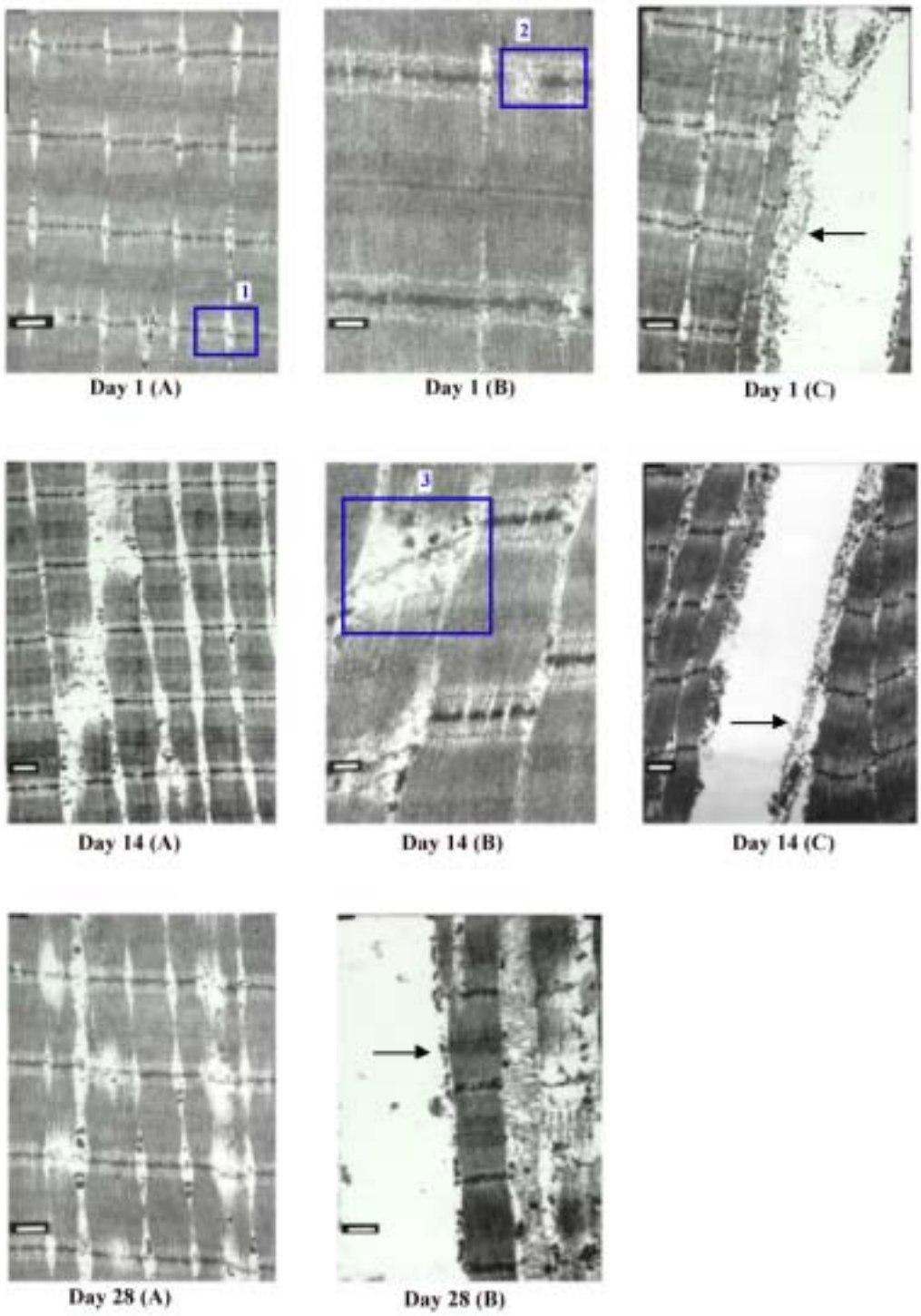

Figure 2. Representative electron microscopic images of normal muscle fibres (i.e., unaffected by electrical stimulation treatment) for 1, 14 and 28 day post-mortem. Rectangles 1 and 2 show disintegration of the attachment between myofibrils and within Z-discs. Rectangle 3 shows degradation of myofibrils. Arrows indicate changes in the sarcolemma during aging. Day 1(A), Day 1 (C), Day 28 (A) and Day 28 (B)=×12,000 (white bars: 500 nm), Day 1 (B)=×30,000 (white bar: 200 nm), Day 14 (A) and Day 14 (C)=×10,000 (white bars: 500 nm), Day 14 (B)= ×25,000 (white bar: 200 nm).

histological studies can be used as an indicator of structural disintegration of the myofibrils with aging.

The effect of electrical stimulation on ultrastructure

Electrical stimulation generally has a beneficial effect on meat tenderness (Hwang and Thompson, 2001a).

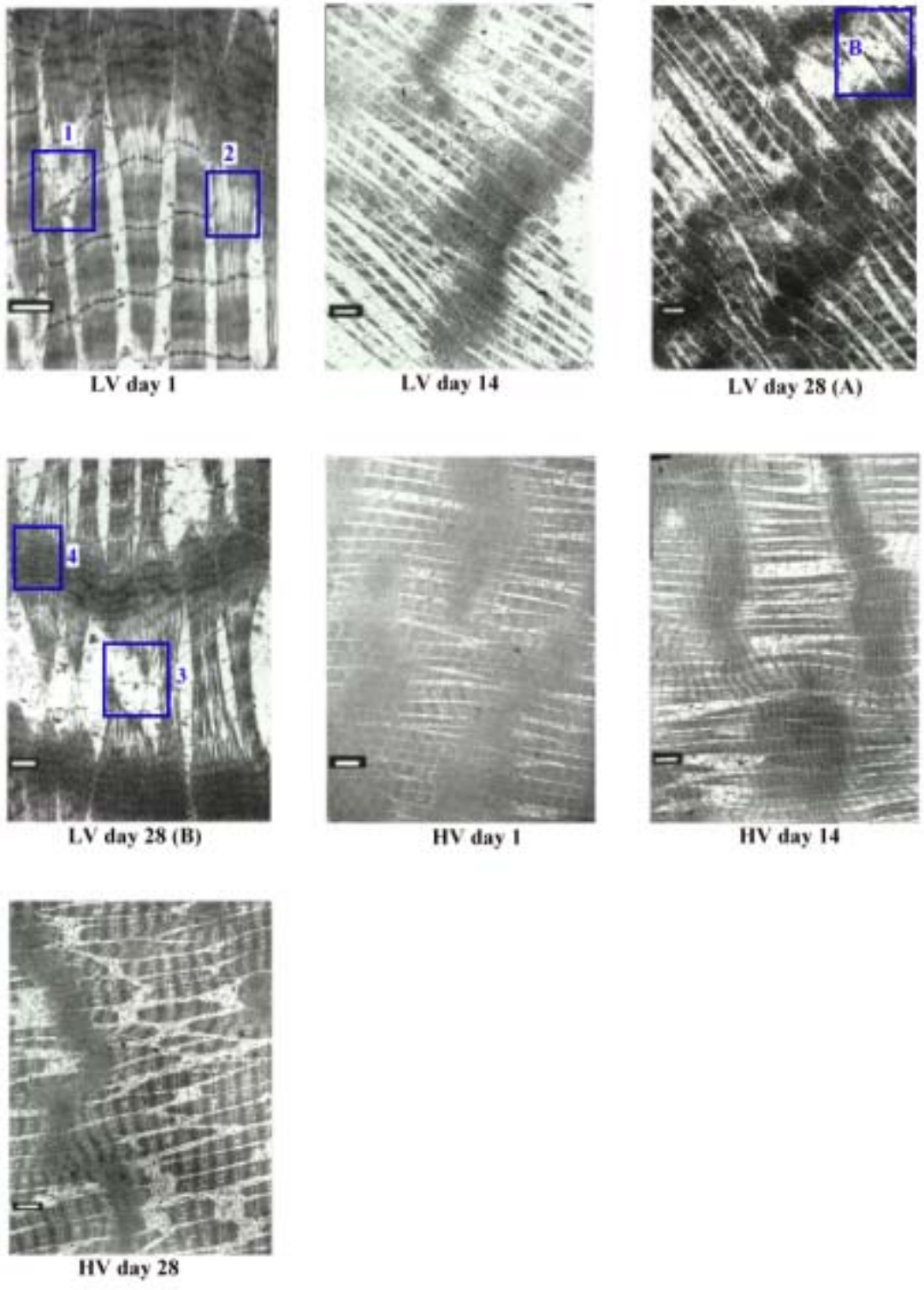

Figure 3. Representative electron microscopic images containing contracture bands for low (LV) and high (HV) voltage stimulation at day 1, 14 and 28 days post-mortem. LV day 28 (B) is the higher magnification of square B in LV day 28 (A). Rectangles 1 and 2 show a tearing and degraded areas adjacent to Z-disc. Rectangles 3 and 4 show differences in degradation of myofibrils between stretched and contracted areas after 28 days of aging. All images=×2,000 (white bar: 2 μm) except LV day 1=×8,000 and LV day 28 (B)= ×5,000 (white bar: 1 μm).

However, some studies showed that inappropriate stimulation procedures resulted in no beneficial (Geesink et al., 1994), or even an adverse effect (Takahashi et al., 1987) on tenderness. The current study showed only a marginal

Table 1. Means (±standard deviation) for pH and temperature at 3 h post-mortem (pH_3 and $Temp_3$), frequency of contracture bands (FCB), percentage of gap areas in longitudinal sections (GLS), WB-shear force, compression and sarcomere length

	Treatment[φ]				Aging (day)[ψ]		
	Cont	HV	LV	LVs	1	14	28
No.	6	6	6	6	8	8	8
pH_3	6.3±0.03	6.0±0.11	5.6±0.04	5.7±0.01	na	na	na
$Temp_3$	23±2.0	22±2.12	24±1.06	25±0.71	na	na	na
FCB (%)	na	89±8.4	67±0.0	42±11.9	65±15	72±25	59±31
GLS (%)	14.3±3.85	14.8±4.08	13.9±3.69	15.4±3.84	10.3±1.89	16.1±2.08	17.5±1.08
WB-shear force (kg)	4.8±1.65	4.1±1.44	4.0±0.86	4.3±0.31	5.2±1.47	4.0±0.67	3.6±0.37
Compression (kg)	2.1±0.31	2.1±0.15	2.1±0.29	2.2±0.17	2.2±0.24	2.2±0.26	2.01±0.16
Sarcomere length (μm)	1.78±0.11	1.79±0.22	1.93±0.18	2.06±0.15	1.84±0.27	1.91±0.20	1.92±0.12

[φ] Means across aging day 1, 14 and 28.

[ψ] Means across the treatments of control (Cont), high voltage (HV), low voltage (LV) and low voltage with slow chilling (LVs) except contrature bands whereby control was excluded.

na: not available for the measurements.

improvement in shear force with stimulation treatment. Certainly using image analysis to quantify the proportion of gaps within and between the myofibril does not reveal any differences, either within stimulated treatments or between the stimulated and control treatments. This does not mean a certain type of stimulation system is not functional, since the effectiveness of electrical stimulation treatment is a function of many factors including voltage, current, time of application, pulse shape, application method, and importantly pre-slaughter animal status.

Although the purpose of this study was focused on quantitative observation of ultrastructural changes, we also counted the number of grids containing contracture bands (table 1). Contracture nodes in electrically stimulated carcass have been suggested as one possible mechanism by which electrical stimulation improved meat tenderness (Dutson et al., 1980; Takahashi et al., 1987). However in the current study high voltage stimulation had a similar shear force to both the low voltage treatments, despite having a higher occurrence of contracture notes. This observation raises a question about the relative importance of physical effects on improving meat tenderness in electrically stimulated carcasses. In general, it has been known that electrical stimulation improves meat tenderness through its effects on prevention of cold shortening, acceleration of the aging process (Hwang and Thompson 2001b), physical disruption of the myofibrillar structure, and alterations in the thermal stability of collagen (Harris and Shorthose, 1988). However, the relative contribution of these components to improvement in tenderness has not been defined. To summarize the current observations, structural deterioration by lateral weakening of the myofibrils and degradation of the Z-disc region occurred as early as 1 day postmortem. The quantitative measurement of the area of gaps between and within myofibrills had potential use in histology studies as a means of quantifying changes in the degradation of myofibre ultrastructure. In addition, the effect of physical impacts of electrical stimulation on meat tenderness was found to be small.

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