# PROTEOLYSIS COMPARISONS OF ELECTRICALLY STIMULATED LAMB

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## Background

There is some evidence that electrical stimulation of lamb carcasses can lead to a faster rate of myofibrillar degradation (HOPKINS & THOMPSON, 2001). As discussed by HWANG et al. (2003) there are two mechanisms which could explain the effect of stimulation on tenderisation. They are a reduction in 'cold-induced' shortening and alteration of protein structure by physical disruption. A secondary effect is the enhancement of the rate of proteolysis potentially stimulated by the release of $Ca^{2+}$ at a higher temperature. To aid the design and commercial adoption of cost effective stimulation systems it is important to understand what effects electrical inputs have on post-mortem muscle.

## Objective

To determine the impact of two different currents on the fragmentation of myofibrillar proteins.

## Methods

*Treatments*

A total of 60 lambs (10 - 11 months of age) were processed at a commercial abattoir. All lambs were second cross suckers from the one property that had been grazing perennial pasture prior to slaughter. Lambs were processed in six blocks of 10. Lambs in blocks 1 & 6 received no stimulation (control). Lambs in blocks 2 & 4 received low current stimulation (constant current 300mA peak, 13Hz or pulses/second, maximum voltage 550V peak). Lambs in blocks 3 & 5 received high current stimulation (constant current 600mA peak, 13Hz or pulses/second, maximum voltage 550V peak) immediately after exsanguination. Stimulation was provided by an on-line system developed by Meat & Livestock Australia.

*Sampling and meat quality measurements*

At regular intervals after the commencement of chilling pH was measured in the left portion of the m. *longissimus thoracis* (LL) at the caudal end over the lumbar/sacral joint. A section of subcutaneous fat and the m. *gluteus medius* was cut away to expose the LL and after measurement the area was resealed with the overlaying tissue. pH was measured using meters with temperature compensation (Jenco Model 6007) and a polypropylene spear-type gel electrode (Ionode IJ 44), calibrated at ambient temperature (16°C). Five to six measurements were taken as the pH declined. When pH 6.0 was achieved a sample of muscle was taken from the cranial end of the LL at the 5th/6th rib. One portion of muscle (~5-10 grams) was frozen at -20°C for determination of myofibrillar fragmentation (MFI) and another portion ~1 gram was frozen in liquid nitrogen for determination of pH. At 24 hours post slaughter, LL muscles from both sides of the carcass were boned and randomly allocated to either a 2 or 4 day ageing treatment and held chilled at ~4.0°C. A sample for determination of MFI's was taken at 1 and 2 days post-mortem and stored frozen at -20°C. The method for determination of MFI's has been previously described was by (HOPKINS et al., 2000). Muscle samples (~1g) held at -70°C were used for determination of pH using iodoacetate. The method was adapted from that described by DRANSFIELD et al. (1992). Samples of the LL and overlying tissue were taken vacuum packed and aged for 2 days and then frozen. These samples were subsequently thawed in a chiller at 4°C, trimmed to 65g blocks of lean and were cooked for 30 min in plastic bags at 70°C in a waterbath. From each block, 6 sub-samples with a cross-sectional area of 1cm$^2$ were tested for shear force. The blocks were cut parallel to the muscle fibres and shear force measured using an Instron Universal Testing Machine with a Warner-Bratzler shear blade fitted.

*Statistical analysis*

pH as determined by iodoacetate and MFI's were analysed using an analysis of variance procedure (GENSTAT, 2002). For the latter trait the fixed effects were treatment (no stim, low or high stim) and post-mortem age of sample (pH 6.0, 1 or 2 days post-mortem). The data was blocked for kill group.

## Results

The rate of decline of pH was similar for the low and high stimulation treatments, which were faster than in non-stimulated carcasses. The overall mean pH ± s.e. for the pH determined using iodoacetate was 6.04 ± 0.03, ranging from 5.55 to 6.38. The mean values ± s.e. and range for the three treatments were 6.15 ± 0.03 (5.91-6.34), 5.92 ± 0.05 (5.55-6.28) and 6.06 ± 0.04 (5.63-6.38) respectively for no stim, low and high stim. These means were significantly different ($P < 0.05$). These sample means indicate that in some carcasses the pH was much lower than the target of 6.0, such that some carcasses were well past the nominal point of rigor (pH 6.0). Because of this and that the objective was to examine the treatment by ageing interaction for MFI's, data for samples with an iodoacetate pH less than 5.8 were removed from the analysis. Data for 5 carcasses from the low stimulation group and for 1 carcass from the high stimulation group were removed.

During ageing there was a significant ($P < 0.001$) increase in mean MFI's (Table 1), but there was no significant effect of treatment on MFI values or a significant interaction between ageing and treatment. The same outcome was found when all carcasses were included in the analysis. The magnitude of the standard error of the treatment means indicates considerable variation.

Table 1. Predicted means (av. s.e.d.) for myofibrillar fragmentation index (MFI) values from a model containing terms for fixed effects (ageing and treatment), including a blocking term for kill group and means for shear force (kg).

| Terms | No. of carcasses sampled | Mean MFI | Av s.e.d. | No. of carcasses sampled | Mean shear force (kg) | Av s.e.d. |
|---|---|---|---|---|---|---|
| Ageing | | | | | | |
| pH 6.0 | 54 | 64c | | | | |
| 1 day | 54 | 95b | | | | |
| 2 days | 54 | 102a | *2.59* | | | |
| Treatment | | | | | | |
| Control | 20 | 86a | | 20 | 4.2a | |
| Low stimulation | 15 | 88a | | 20 | 3.4b | |
| High stimulation | 19 | 89a | *6.41* | 20 | 3.5b | *0.16* |

Means followed by a different letter in a column (a, b, c) are significantly different ($P < 0.05$).

## Discussion

Given that stimulation (high and low) reduced shear force after 2 days of ageing compared to non stimulated meat it may have been expected that a similar response would be found for MFI values. This was not the case and there was no interaction between ageing and treatments. Interestingly under controlled conditions HOPKINS & THOMPSON (2001) found that low voltage stimulation did accelerate the increase in MFI's during ageing indicative of increased myofibrillar degradation, but the stimulation treatment had no impact on reducing shear force. GEESINK et al. (1994) found that the length of the stimulation time affected the response and interactions between stimulation and chilling temperature can impact on the response observed through the impact of the chilling regime on sarcomere length. For example GEESINK et al. (2001) reported that high-voltage stimulation reduced shear force, in spite of no effect on proteolysis, but in which case control muscle had significantly shorter sarcomeres. By contrast when there was no difference between treatments for sarcomere length and stimulated muscle had lower shear force values, this was mirrored by an increased change in MFI for *longissimus*, but not *semimembranosus* muscle (GEESINK et al., 1993). The relative importance of proteolysis and sarcomere length for the current samples can not be established, as no measures of sarcomere length were available. However it appears based solely on MFI values that the improvement in shear force was not due to an increase in myofibrillar degradation. So the question remains was it simply that the stimulation treatments reduced the impact of cold induced shortening, giving rise to an improvement in shear force after 2 days of ageing?

The variation of MFI values for treatment means was higher than reported previously (eg HOPKINS & THOMPSON, 2001) which indicates greater differences between samples within treatments. This also contributed to the fact that no significant difference was found between treatments. The time of sampling for the nominal pH 6.0 samples was based on measurement of pH at the caudal end of the loin muscle and sampling was at the cranial end for determination of pH using iodoacetate. This partially explains why some carcasses had to be excluded from the analysis. It is also likely that by the time a carcass was sampled the pH had dropped significantly from when the nominal pH 6.0 value was measured. Measurement and sampling at the same site in the loin would have reduced this effect. The large increase in MFI values with ageing is as expected reflecting increased fragility of the myofibrillar proteins a widely reported response.

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