Comparison Of Different Types Of Low Voltage Electrical Stimulation On Tenderisation: Interaction With Stress And Calpains

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Introduction

Pre-slaughter stress has long been associated with a deterioration in meat quality. It has been proposed that stress lowers pre-slaughter muscle glycogen levels which in turn leads to carcasses of high ultimate pH (Gregory, 1996). However, the relationship between pH and meat quality is complex. Red meat with an ultimate pH of 5.8 to 6.2 is tougher than meat of higher or lower ultimate pH (Watanabe *et al.*, 1996). Other research has related the rate of pH fall to tenderness (Marsh *et al.*, 1987). However, there is no evidence that pH has a direct role in meat tenderisation. One possible hypothesis is that pH operates by altering the rate of calcium-dependent proteolytic breakdown of the myofibrils by the calpains (Dransfield, 1993).

Another aspect of meat processing that impacts on meat tenderisation is the application of electrical stimulation. The New Zealand meat industry introduced high voltage electrical stimulation (HVES) to avoid the problems of cold shortening. Sheep carcasses are also subjected to low voltage electrical stimulation (LVES) immediately after slaughter. This allows more rapid dressing of the carcasses. However, LVES may also affect tenderisation. We have previously identified an interaction between LVES, stress and tenderness in sheep (Bickerstaffe *et al.*, 1996). There is evidence that the effect of electrical stimulation on tenderisation varies with the voltage and duration applied. Anecdotal evidence suggests that different processing plants vary in their proportion of unacceptably tough carcasses. Unlike HVES, LVES is not standardised and thus may be a cause of the plant-to-plant variation.

Objective: To study the interaction of electrical stimulation, stress and tenderisation in sheep.

Methods

A flock (n=230) of Romney lambs was divided between two export processing plants. At each plant the flock was further divided into three groups based on the level of preslaughter stress. Low stress (50 animals) was defined as swim washing on arrival at the processing plant and then held next to the processing ramp. The next day these animals were slaughtered first. Moderate stress (50 animals) involved holding the lambs in the centre of the yards overnight, swim washing them a second time 3h before slaughter and using dogs to assemble the mob. The control group was left unwashed to provide minimal stress (20 animals at plant A and 10 at plant B).

Immediately following slaughter half of the carcasses from the control groups and 30 carcasses from each of the other groups were subjected to LVES. At plant A this was Spinal Discharge (480Vpk for 10 sec) and at plant B, Low Voltage Immobilisation (80Vpk for 30 sec). All carcasses were subjected to HVES (1130Vpk 90 sec) within 30 min of stunning. Carcasses were chilled at 15°C for 8h, 10°C for 4 h and 1°C for 15h. After 27h, the carcasses were boned out and the LD muscle vacuum packed and held at -1°C for 2 weeks.

The temperature and pH of the *Longissimus dorsi* (LD) muscle was measured on all carcasses using an Orion 8163 spear pH electrode immediately before HVES and at intervals up to 24 hours after slaughter. LD muscle steaks (45mm) were taken for tenderness determination from 10 carcasses in each group at 4h, 12h, 1 day, 4 days, 7 days and 14 days. The tenderness of the meat was measured, after it had been cooked to an internal temperature of 75°C, using a MIRINZ tenderometer (Devine and Graafhuis, 1995).

Biopsies (5g) for enzyme assays were taken from the LD of 8 stimulated and 8 unstimulated carcasses in the low stress group. The calpains were separated from calpastatin using a DEAE-sepharose ion-exchange column and their activity assayed using casein proteolysis (Koohmaraie, 1990). A unit of calpain was defined as the amount which gave an increase in A_{278} of 1.0 in 1 hour. Calpastatin activity was determined by its ability to inhibit m-calpain.

Statistical analysis was carried out using the Minitab computer package, version 10.1.

Results

A comparison of the two plants (Table 1) showed that meat from plant B was slower to tenderise (significantly tougher at 4 and 12h post-mortem) and remained significantly tougher following aging ($p<0.01$) for 14 days. This meat also had a higher pH at both 1.5h post-mortem and ultimate pH ($p<0.01$). However the average pH and tenderness values from both plants were acceptable.

Table 1. Comparison of shear force and pH from the two plants.

Plant	N	kgF 12 hours	kgF 14 days	pH 1.5hours	pH ultimate
A	120	5.73	3.26	5.96	5.66
B	110	6.49	3.69	6.02	5.73
Significance		p<0.05	p<0.01	p<0.01	p<0.01

When the results from the two plants were analysed for the effect of stress, those animals which were subjected to minimal and low stress produced carcasses that were more tender than those exposed to moderate stress. At plant A this stress effect was associated with a slower rate of pH decline ($p<0.01$) and at plant B it was related to higher ultimate pH ($p< 0.01$).

Table 2. The effect of stress on pH and shear force.

Plant	Stress	kgF 14 days	pH 1.5 hours	pH ultimate
A	minimal	3.18 [a]	5.79 [a]	5.65
	low	3.17 [a]	5.79 [a]	5.66
	moderate	3.46 [b]	6.19 [b]	5.68
B	minimal	3.53 [b]	6.05	5.68 [a]
	low	3.52 [b]	6.04	5.69 [a]
	moderate	3.94 [c]	6.00	5.79 [b]

Numbers from the same column and plant with different superscripts are significantly different ($p<0.05$).

Overall the electrical stimulation immediately post-slaughter had no significant effect on tenderness at either works, however there appeared to be an interaction with stress. At plant A LVES was associated with tougher meat from the moderately stressed animals while at plant B it was meat from those animals which had been exposed to low stress. In both cases LVES had no effect on pH at 1.5h or on the ultimate pH.

Table 3. Effect on shear force of the interaction of electrical stimulation and stress.

Plant	Stress	LVES	kgF 4 h	kgF 12 h	kgF 1 day	kgF 4 day	kgF 7 day	kgF 14 day
A	High	No	7.70	5.11	5.66	4.11	3.89	3.49
		Yes	7.39	5.80	5.99	4.73	4.34	3.44
B	Medium	No	8.46	5.51	3.95 [a]	3.56	3.77	3.31
		Yes	9.17	6.56	5.51 [b]	4.18	4.30	3.72

Numbers from the same column and plant with different superscripts are significantly different ($p<0.05$).

The levels of the calpains and their inhibitor calpastatin were assayed in 8 carcasses from each of the moderately stressed groups. There was no significant difference in the amount of calpains either immediately before HVES or at 12 hours post-mortem. However the meat from the moderately stressed animals from plant B had significantly higher ($p< 0.01$) levels of inhibitor at 12 hours.

Table 4. Post-mortem changes in the activity of the calpain system (units/gram) in the *Longissimus dorsi*.

Plant	LVES	μ-calpain		m-calpain		calpastatin	
		25 min	12 h	25 min	12 h	25 min	12 h
A	no	1.26	0.38	1.67 [a]	2.00	3.88	1.09 [a]
	yes	1.28	0.34	1.71 [a]	1.88	3.18	1.34 [a]
B	no	1.14	0.30	1.18 [b]	1.76	2.78	1.03 [a]
	yes	1.33	0.37	1.67 [a]	1.81	3.37	2.25 [b]

Numbers from the same column with different superscripts are significantly different ($p<0.05$).

Discussion

Our results confirmed earlier work that subjecting animals to preslaughter stress led to tougher meat and that this was associated with pH changes (Purchas and Aungsupakorn, 1993). However we have also confirmed our earlier results of a pH-independent interaction between stress and LVES (Bickerstaffe *et al.*, 1996). There was evidence that this toughening effect might be mediated through the calpain system. As calpastatin is a substrate for μ-calpain, the higher level of calpastatin remaining in the group where LVES toughened the meat may be an indicator of μ-calpain working less efficiently in these carcasses (Dransfield, 1993). Comparison of the two plants showed that the plant B produced significantly tougher meat. The pH data indicates that higher stress may be the cause of the difference. The lack of a direct measure of stress has made it difficult to determine how the different stress treatments affected the animals i.e. was low stress at plant B equivalent to moderate stress at plant A?

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