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Review

Reassessing the principles of electrical stimulation

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ABSTRACT

The mechanisms by which electrical stimulation (ES) of carcasses can be used to modulate meat quality are reviewed. Evidence to support an effect of ES on tenderness (and other meat quality attributes) based solely on changes in the pH/temperature profile within carcass muscles are presented. The interactions between electrical parameters and the contraction responses of carcass muscles are described to provide generalised principles to guide the design of electrical stimulation technology. The commercial risks to meat quality of inappropriate use of electrical stimulation, particularly excessive stimulation to produce PSE-like conditions, are considered.

Contents

1. Introduction

Electrical stimulation of carcasses is now a standard meat processing technology, used primarily in the beef and lamb industries. The main commercial incentive is to accelerate the tenderisation of meat, although a range of other meat quality attributes are also affected by this carcass intervention, including meat colour, colour stability and water binding properties. The objectives of this review are to evaluate the various forms of electrical stimulation and their effects on meat quality, identify the commercial opportunities that are as yet largely unexplored and identify future opportunities and developments.

2. Effects of electrical stimulation on meat tenderness

The obvious consequence of applying an electrical current to a carcass is to induce vigorous muscular contractions and, in

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response to the increased energy expenditure, cause a dramatic acceleration of pH decline. Electrical stimulation (ES) of a beef carcass can routinely drop the muscle pH by 0.5 units over a period of 60 s of stimulation, a process that could require three or more hours in the absence of stimulation (Ducastaing, Valin, Schollmeyer, & Cross, 1985). This represents a 180-fold acceleration in the rate of muscle glycolysis and a clear indication of the tight coupling between the rate of glycolysis and ATP turnover in muscle tissue. In addition to the pH drop during stimulation (ΔpH), there can also be an acceleration in the subsequent rate of glycolysis following stimulation ($\Delta pH/\Delta t$) above that seen in unstimulated muscles (Chrystall & Devine, 1978). However, this effect tends to be associated with high voltage stimulation (HVS) systems (typically 1000 V and above) and is not necessarily triggered by low voltage stimulation (LVS) systems (Kastner et al., 1993).

2.1. Does electrical stimulation induce tenderisation through muscle damage?

Marsh (1986) and Takahashi et al. (1987) demonstrated that electrical stimulation with a 60 Hz waveform produced myofibrillar damage in muscle tissue, described as areas of supercontractions and associated areas of myofibrillar stretching. They provided evidence that a small pH decline following stimulation with a 60 Hz waveform produced greater tenderisation than a large pH decline using 2 Hz waveform. Since the latter did not cause any myofibrillar damage, the authors concluded the key mechanism of ES induced tenderisation is via direct myofibrillar damage rather than the effect of an accelerated pH decline.

The short time intervals between pulses when stimulating with high frequencies ($\geqslant$30 Hz) prevents effective sequestration of the released calcium so that high levels of intracellular calcium accumulate, the contractions become fully fused and maximal contraction forces are generated (Bagshaw, 1993). The high contraction forces are, presumably, the ideal conditions to produce structural damage to the myofibrils. However, it is interesting that more recently, Ho, Stromer, and Robson (1996) reported that contraction nodes could be found after stimulation at 20 Hz, which produces nearly completely fused contractions but not at the same peak forces as are seen with higher frequencies stimulation (Wilson, 1972).

In addition to possible direct effects of stimulation on the structure of the myofibrils, the possibility that calpain-induced proteolysis can be activated directly by calcium released during stimulation has been suggested (Hwang, Devine, & Hopkins, 2003). Presumably, the electrical stimulation would need to produce highly exaggerated free intracellular calcium levels compared with those produced by normal physiological muscle activity in vivo since, otherwise, exercise would have limited health benefits! We therefore explored the effects of a range of different stimulation waveforms to assess any acceleration of tenderisation relative to a conventional 15 Hz waveform employed in New Zealand. The assessment was carried out in a commercial lamb abattoir using their normal carcass chilling regime, and any effects on tenderness were based on shear force measurements of *m. longissimus* 24 h after slaughter. The stimulation was carried out either immediately after slaughter in the undressed carcass or at 30 min after slaughter. The stimulation was supplied via a constant current stimulation unit (Applied Sorting Technologies, Victoria, Australia) that was set to deliver 1 A for 60 s using manual electrode clips that were applied to the neck and anus. In total, 800 lamb carcasses were used for these trials over a period of several weeks.

Of particular interest was whether or not increasing the stimulation frequencies could accelerate tenderisation independent of any changes in the rate of pH decline or, alternatively, that reduced frequencies were less effective. As a further experimental variable, this work also considered a possible effect of eccentric contraction – forced extension of the muscle while in a stimulated, contracted state – which is known in vivo to increase the physical stress on muscle tissue and cause disruption of muscle fibres and muscle soreness (McCully & Faulkner, 1986). Although the various stimulation protocols produced a significant pH decline and improvement in tenderness at 24 h compared with unstimulated carcasses, a consistent effect of either stimulation frequency and/or eccentric contractions was not found (Fig. 1). We are therefore unable to confirm a direct effect of stimulation waveform or frequency as a mechanism for tenderness changes following electrical stimulation.

2.2. Is the effect of ES to prevent cold-shortening?

Electrical stimulation was developed originally in New Zealand in the late 1970s to manage toughening in lambs that were being frozen rapidly after slaughter, an extreme cooling regime that clearly produced cold-shortening and associated toughening. The need to process large numbers of lamb for export drove this

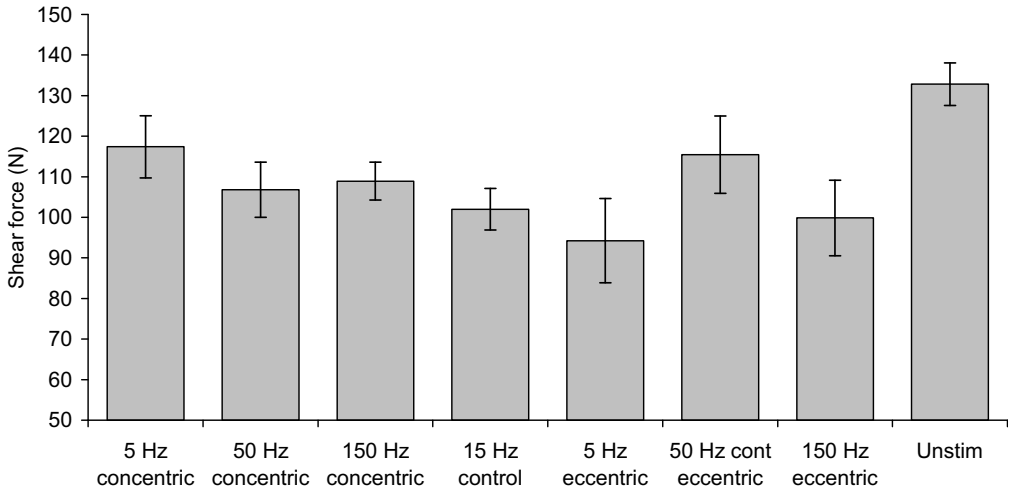

Fig. 1. The effect of different stimulation frequencies and carcass restraint during stimulation on the shear force of lamb *m. longissimus* samples measured at 24 h post-mortem. Shear force was measured on cooked samples using a Tenderometer. The values given are the mean (SE) for each treatment. There were 30 samples per treatment.

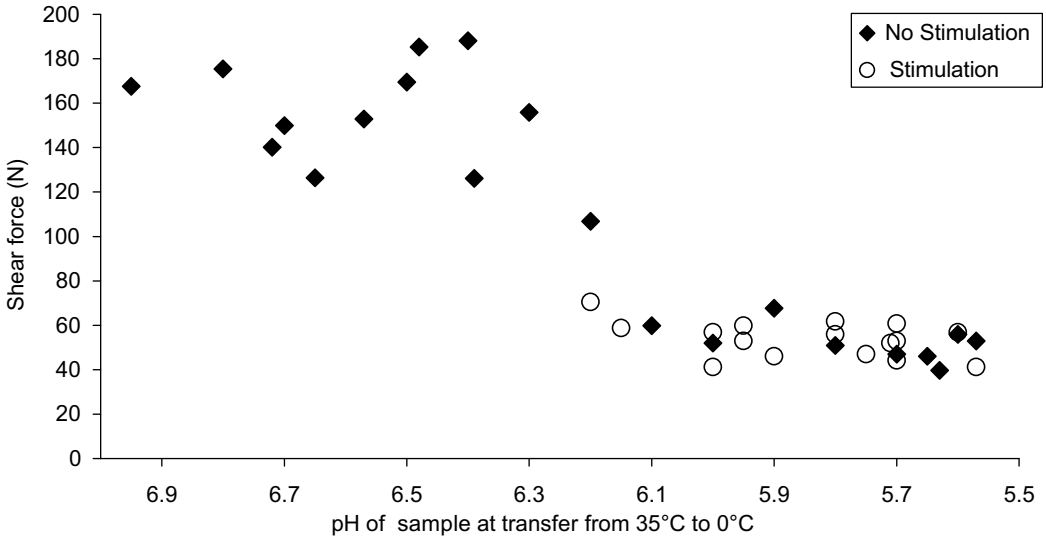

Fig. 2. Shear force of beef *m. longissimus* samples following pre-rigor incubation at 0 °C and 3 weeks of ageing. Shear force was measured on cooked samples using a Tenderometer. Each value is the mean of four samples.

practice, but it is questionable whether current processing practices are likely to resort to such practices.

Cold-induced contracture can be measured at temperatures as high as 25 °C, although its severity increases as the temperature falls further (Davey & Gilbert, 1975; Honikel, Roncales, & Hamm, 1983), so some level of cold-shortening is likely to be commonplace in carcass processing. But the true benchmark of cold-shortening, and its only commercial significance, is persistent toughening, and this requires a level of contracture in excess of 20% of rest length (Davey & Gilbert, 1967). It is also widely recognised that the contracture is ATP dependent and its extent will therefore decrease with the muscle pH (Honikel et al., 1983); for example, Scopes (1971) demonstrated in lamb an almost linear relationship between the magnitude of contraction and pH, and the effect disappears completely at pH 6. This relationship between temperature and pH has led to the generalisation that cold-induced toughening is avoided as long as the temperature is above 10 °C until the pH is below 6.

To re-evaluate the conditions needed to produce cold-induced toughing and as part of a process of modelling the cold-shortening phenomenon, we transferred pre-rigor beef *m. longissimus* from 35 °C to a 0 °C waterbath at various pre-rigor pH's and measured the effects on tenderness after 2 weeks of ageing at 0 °C. The results show that to avoid persistent cold-induced toughening when the sample temperature is reduced to 0 °C, the muscle pH had to be below pH 6.2–6.3 (Fig. 2). This result shows that faster chilling is possible without risk of toughening than the requirement that the pH should be below pH 6 before the temperature falls below 10 °C. To generate cold-induced toughening by this new criterion in a beef carcass – or even a lamb carcass – would require extreme chilling conditions and is unlikely to exist in most commercial processes.

3. The effects of electrical stimulation on tenderness are mediated directly by pH decline

In the absence of any clear evidence for tenderisation through modifications of muscle structure, and the unlikely occurrence of cold-induced toughening under normal processing conditions, the remaining explanation for the effects of electrical stimulation on tenderness is based solely on the accelerated pH decline (Chrystall & Devine, 1985; Chrystall & Hagyard, 1975; George, Bendall, & Jones, 1980). This mechanism is based on the principle that the tenderisation process begins at or near *rigor mortis*, when the ultimate pH is attained. Since electrical stimulation accelerates the onset of *rigor mortis*, tenderisation begins sooner and, more importantly, this earlier onset of *rigor mortis* is associated with a higher carcass temperature so the rate of tenderisation is faster than would be the case if *rigor mortis* is attained later and the carcass temperature is lower. Of course, the carcass temperature would eventually decline to normal chill temperatures, but by then, the stimulated carcass will have had a head start. By this scenario, the ultimate tenderness attained will be the same for both the stimulated and unstimulated carcass (assuming the stimulation is not excessive, in which case the final tenderness will be less in the stimulated carcass; this will be addressed later), but the stimulated carcasses will reach the final tenderness sooner than the unstimulated counterpart (Chrystall & Daly, 1996).

The benefits of accelerated pH decline can be illustrated through a calculated tenderness curve. Using published data on the temperature dependency of ageing (Dransfield, Jones, & MacFie, 1980; Graafhuis et al., 1992), the effects of onset of *rigor mortis* at 2 h in a stimulated carcass can be compared with *rigor mortis* at 12 h in an unstimulated, using an identical and representative carcass chilling curve (Fig. 3). This calculation clearly identifies that less tenderisation occurs in the unstimulated carcasses during the first week of ageing, without any need to invoke a cold-shortening phenomenon.

It is possible therefore that a slower ageing rate caused by a faster chilling rate is misinterpreted as a toughening effect caused by cold-shortening. For example, in a trial where beef animals were processed without any electrical interventions and the carcasses placed into 0 °C temperatures within 30 min of slaughter, the *m. longissimus* took more than 30 days of ageing before it attained shear force values that were deemed acceptable by consumers, but the results show that the persistent toughness associated with cold-shortening did not occur, even though tenderness measurements carried out before 30 days could be misinterpreted as such (Simmons, Auld, Mudford, & Nagle, 1999).

This explanation for the benefits of electrical stimulation on meat tenderness therefore claims that it is not the rapid pH fall per se that accelerates tenderisation but rather the interaction of the faster pH decline and temperature. This argument would

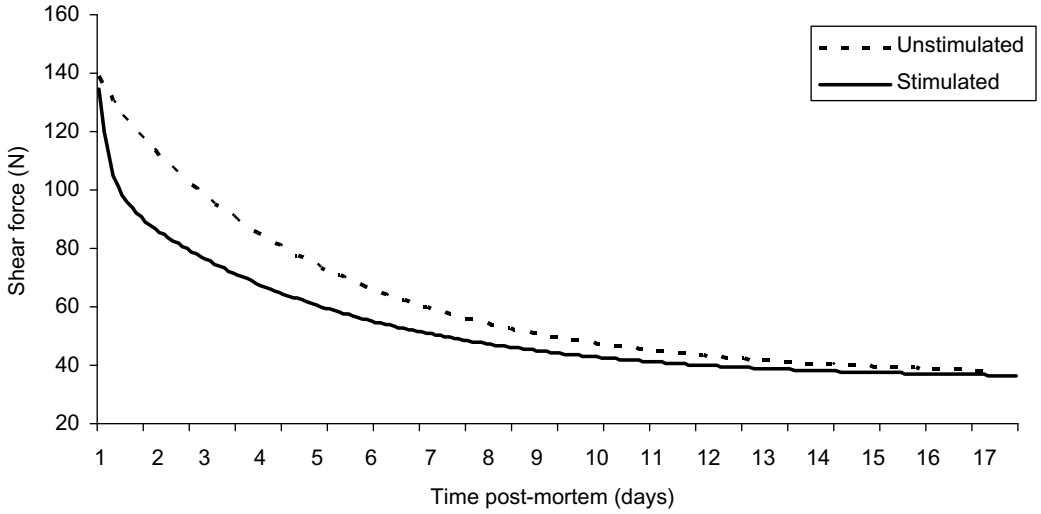

Fig. 3. Calculated tenderness curves for stimulated and unstimulated carcasses.

Table 1
The effect of stimulation followed by incubation at four different temperatures during the pre-rigor period on the shear force of beef *m. longissimus* samples

Temperature (°C)	1 day post-mortem		5 days post-mortem	
	Electrical stimulation	No stimulation	Electrical stimulation	No stimulation
15	123.6	134.9	52.9[a]	55.0[a]
25	128.5	130.1	54.2[a]	55.0[a]
37	126.3	121.0	62.9[b]	59.3[a]
40	137.6	141.6	83.1[b]	78.7[b]
SEM	2.19	3.13	2.42	2.28
p-value	ns	ns	***	***

Within a column, means with a different letter are significantly different ($p < 0.05$). There were no statistical differences between the stimulation and no stimulation treatments at the different temperatures at either 1 day or 5 days post-mortem.

predict that maintaining constant temperatures in stimulated and unstimulated muscles during the pre-rigor period and indexing the first shear force measurement to *rigor mortis* would remove any effect of ES on tenderness. This expectation was confirmed by Martin, Hopkins, Gardner, and Thompson (2006), who incubated muscles at 15 °C immediately following stimulation. The results in Table 1 used a similar methodology but also considered the possibility of an interaction between electrical stimulation and a range of higher incubation temperatures, since stimulation will create a condition of low pH and high temperature in a muscle in situ during the pre-rigor period. The *m. longissimus* from four beef carcasses were removed from one side without prior stimulation while the contralateral side was removed immediately following electrical stimulation with a 15 Hz waveform using 10 ms and 500 V pulses. The pH's were 6–6.2 immediately following stimulation. Following removal, the muscles were incubated at either 15, 25, 37 or 40 °C until *rigor mortis*, then transferred to 15 °C for ageing for 5 days. An effect of stimulation on shear force could not be detected at any of the incubation temperatures, either at *rigor mortis* or after 5 days of ageing. However, the incubation temperature did have an effect on the shear force value, an effect that will be discussed further in a later section.

A key component of this explanation on the effects of ES is the requirement that the tenderisation process begins at or near *rigor mortis*. The ultimate pH represents the final cessation of glycolysis and, hence, the final loss of cellular ATP. The assumption that tenderisation begins at ultimate pH has not been rigorously tested and it may be that a specific ATP concentration identifies when many cellular functions, such as calcium sequestration, break down and calcium-dependent proteolysis is activated. For example, Simmons, Singh, Dobbie, and Devine (1996) demonstrated that calpains begin to show signs of autolysis at pH around 6–6.2, which, presumably, reflects a calcium-mediated event. But this does not alter the key requirement of the effect of ES on tenderness, which is that the events triggering proteolysis occur sooner – but through the same mechanism – as is seen in muscle that is not electrically stimulated.

4. Electrical parameters required to produce electrical stimulation

The use of electrical stimulation to manipulate muscle contractions and therefore pH decline can be effectively exploited given an understanding of the interactions between the electrical parameters (pulse amplitude, width and frequency) and the magnitude of the muscle contraction they produce. To study this relationship in beef and lamb carcasses, we developed a methodology to measure muscle contraction forces based on recording intramuscular pressure (Daly, Simmons, & Mudford, 2000). This technique inserts a 16 g catheter into the muscle and delivers through it a low flow of saline (0.3 mL/min). The saline flow ensures that the muscle tissue does not block the end of the catheter and allows the saline to act as the pressure transducer. A pressure sensor in series with the catheter measures the changes in intramuscular pressure during contraction.

4.1. Do responses to low voltage stimulation require the nervous system?

One application of the muscle pressure measurement was to re-evaluate the question of how the LVS systems – typically 100 V or less – generate a muscle contraction. Bendall (1976) argued that stimulation requires a patent nervous system to mediate the muscle response since prior treatment with a neuromuscular blocking agent, pancuronium, abolishes muscle contractions in response to stimulation using 200 V.

However, Shaw, Eustace, and Warner (1996) reported an effective pH drop using LVS applied 25–30 min post-mortem and we also reported the same based on commercial lamb systems in New Zealand (Simmons, Gilbert, & Cairney, 1997). We therefore

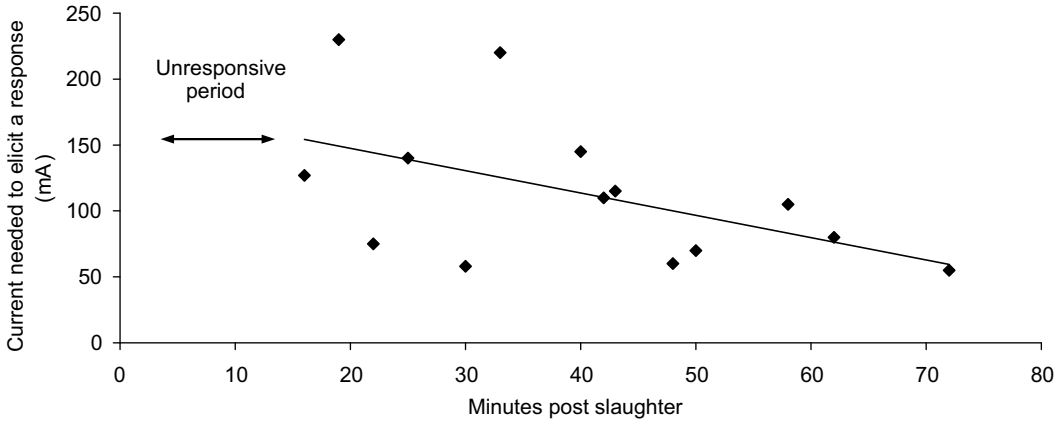

Fig. 4. Emergence of responsiveness to stimulation in the *m. longissimus* of curare-treated lamb carcasses.

re-evaluated the role of the nervous system during LVS immediately following slaughter using sheep carcasses treated with curare. These were stimulated using a current-controlled (Applied Sorting Technologies, Victoria, Australia) 15 Hz stimulation waveform delivering a 0.5 A pulse amplitude (10 ms pulse width), and were unresponsive to stimulation for approximately 10 min following the stun. Beyond this time, a response reappeared and increased in strength over a further period of 5–10 min (Fig. 4). This recovery of responsiveness was not due to a loss of the neuromuscular block because direct stimulation of the femoral nerve could not elicit a response in the *m. semitendinosus* at a time when direct stimulation of the carcass could. The explanation for this phenomenon is not clear but demonstrates a significant drop in the threshold current needed to produce a response during the early post-mortem period. The result therefore confirms that LVS is mediated by the nervous system while it is functional but, subsequently, can evoke direct muscle stimulation through changes in muscle membrane excitability.

4.2. Dose–response attributes of carcasses to electrical parameters

Traditionally, there have been two primary options for electrical stimulation: LVS, normally applied during bleeding and employing voltages of less than 100 V peak; and HVS, normally applied after dressing and employing up to 1143 V peak. Both these systems use 10 ms pulse durations. Research reports from the USA frequently make reference to waveforms derived from 60 Hz mains waveforms, usually delivered as intermittent bursts (for example, on:off cycles of 2:1 s) and a voltage in the 200–300 range (Ducastaing et al., 1985). More recently, a system developed in Australia by Meat and Livestock Australia, uses an intermediate voltage (300 V peak) but a unipolar pulse duration of 1 ms (Meat Technology Update, December 2007). These varying electrical parameters produce a range of options and their relative benefits for meat quality, operator safety and installation costs need to be clarified.

A generalised description of the effects of pulse width and pulse amplitude on muscle responses can be formed from established physiological principles. For a given pulse amplitude, reducing pulse width weakens the response until it disappears altogether (Aston, 1991). However, pulse width and pulse amplitude are interchangeable: what is lost by reducing the pulse width can be regained by increasing the pulse amplitude, the limit being the pulse width that requires such a large voltage that tissue damage occurs through ohmic heating. These principles can be described in the classic pulse strength–duration curve which defines the threshold for producing an electrically induced stimulus. A calculated pulse strength–duration curve taken from Aston (1991) is shown in Fig. 5.

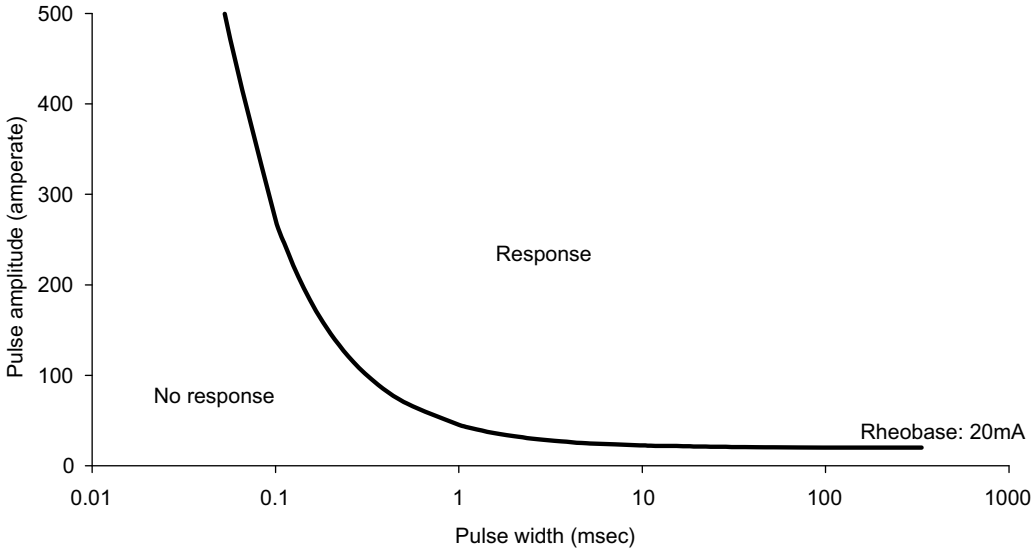

Fig. 5. Pulse strength–duration curve to define the threshold for a response to an electrical stimulus.

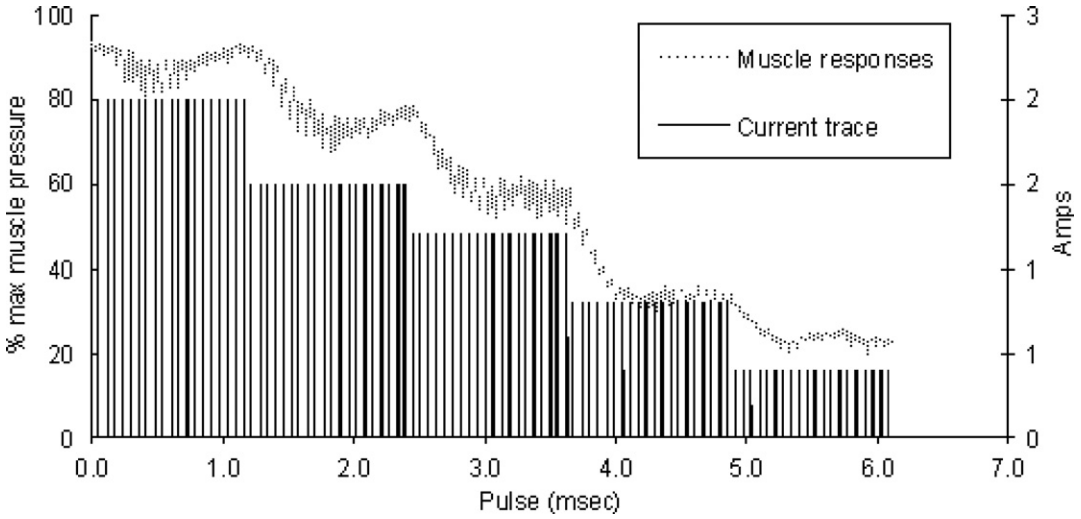

Fig. 6. Muscle pressure response in the *m. longissimus* of a lamb carcass during stimulation. Stimulation pulses modulated to produce a step-wise increase in pulse amplitude; within each step four different pulse widths (0.5, 1, 5, 10 ms) were delivered.

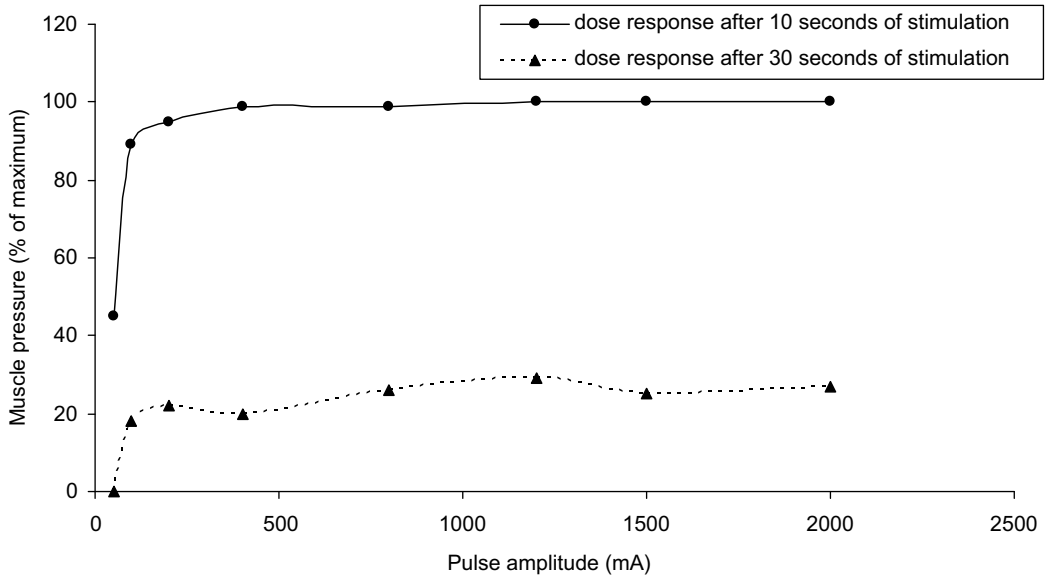

Fig. 7. Dose–response of lamb to electrical stimuli immediately after slaughter.

We have used measurements of muscle pressure to put actual values to the relationship between electrical parameters and muscle responses in stimulated lamb carcasses. To do this, a constant current stimulation unit delivered electrical pulses of defined amperage and pulse width that were used to stimulate the *m. longissimus* in lamb carcasses. The magnitude of the responses was measured by the intramuscular pressure changes. The pulses were modulated to produce a step-wise increase in pulse amplitude, from 50 to 300 mA and, within each step, five pulses each of 0.5, 1, 5 and 10 ms were delivered. Fig. 6 shows a segment of a pulse train of descending pulse amplitude, together with the associated muscle pressure measurements.

Electrically stimulating carcasses immediately after slaughter (early stimulation) produces a number of distinctive characteristics (Fig. 7). The responses are insensitive to pulse width (at least within the range used in the experiment – data not shown) and the threshold current amplitude needed to produce a muscle contraction is very low (less than 50 mA). Furthermore, the dose–response (increase in force per unit per increase in stimulation magnitude) was very steep, reaching a maximum at less that 100 mA and demonstrating something close to an 'all or nothing' response characteristic. However, continuous stimulation caused the responses to decay rapidly so that, after 30 s of stimulation, they were reduced to approximately 20% of the responses after 10 s of stimulation. We conclude that these results are characteristic of muscle contraction responses mediated by the nervous system.

This contrasts to the response characteristics measured in carcases after dressing (20–30 min after slaughter; late stimulation). Fig. 8a shows a more defined force increase in response to increased stimulus strength. This wide distribution of threshold voltage undoubtedly reflects the range of fibre diameter, the main determinant of electrically induced depolarisation threshold for both nerve and muscle tissue (Daly, 2005).

Also, an important influence of pulse width becomes evident as the carcass becomes increasingly fatigued and the pH falls. At the outset, changing pulse width produced little effect within the range used in these experiments. But, as the carcasses became

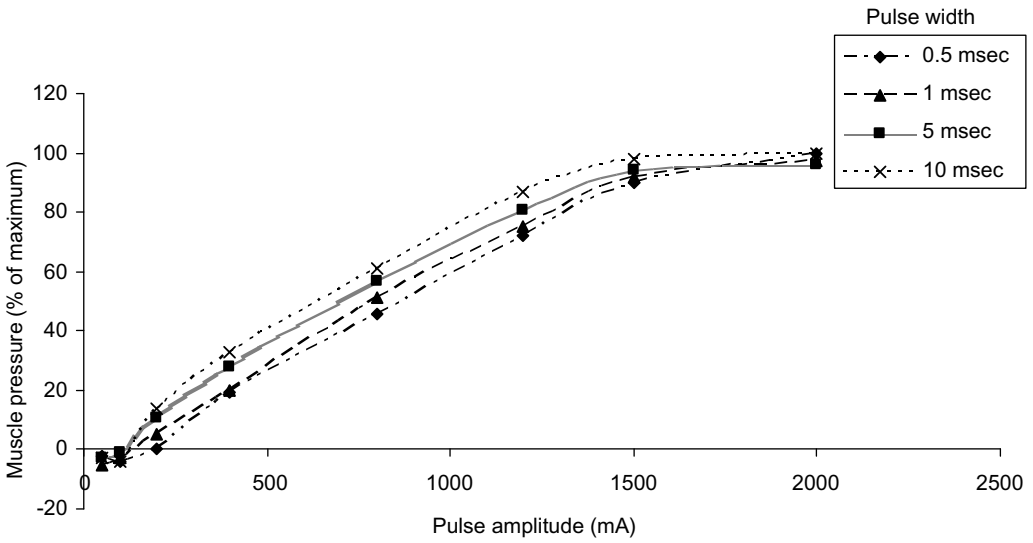

Fig. 8a. Dose–response to electrical stimuli after 10 s of stimulation in a lamb carcass post-dressing; responses during first 10 s of stimulation.

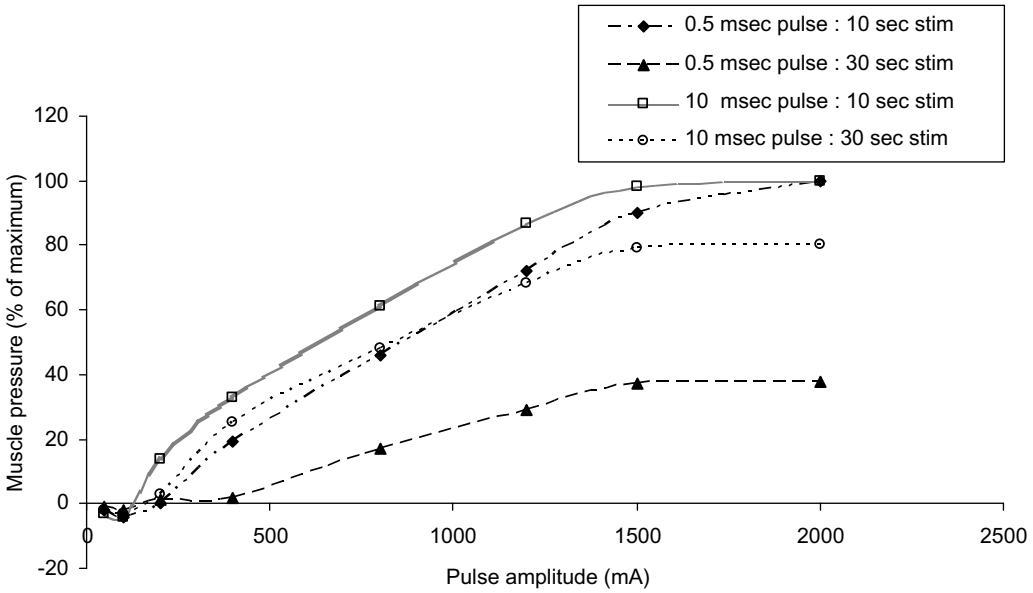

Fig. 8b. Dose–response to electrical stimuli in a lamb carcass post-dressing: comparison of responses after 10 or 30 s of stimulation.

increasingly fatigued, the threshold for responses increases and wider pulse widths became more effective at maintaining a response (Fig. 8b).

An additional effect needs to be accounted for when considering stimulation frequency. Fig. 9 shows the response characteristics from the *m. longissimus* of sheep carcasses stimulated at either 15 Hz or 50 Hz. The higher frequency produced a higher peak contraction force, as would be expected by the fully fused, tetanic contraction. However, the response decayed much faster, an effect that cannot be attributed solely to the higher metabolic demand of the greater force generation. The area under the curve, a measure of the work done by the muscle and related, therefore to the pH decline, is 35% less in the 50 Hz stimulation compared with 15 Hz (after normalising the measured force to the maximal contraction force generated from each muscle during a 1 s, 50 Hz test stimulation). This phenomenon probably explains the lesser pH decline produced by stimulation with continuous high frequencies (Chrystall & Devine, 1978). The explanation can probably be attributed to the accumulation of extracellular potassium in response to the rapid rate of stimulation that renders it refractory to further stimulation (Cairns, Flatman, & Clausen, 1995; Juel, 1988). However, short periods of rest allows the membrane to recover and so, by using an intermittent stimulation waveform, an effective pH decline is possible with high frequencies. Ultimately, the muscle becomes unresponsive due to metabolic fatigue and extreme pH decline.

4.3. Physiological responses to stimulation

While the electrical parameters define the stimulus, the physiological characteristics of the muscle define the contraction responses. For example, an obvious variable in the response characteristics of a muscle will be the fibre type composition, which will define the extent of calcium release and its rate of reuptake, together with the properties of the associated force generation is specific to the fibre type composition (De Luca, LeFever,

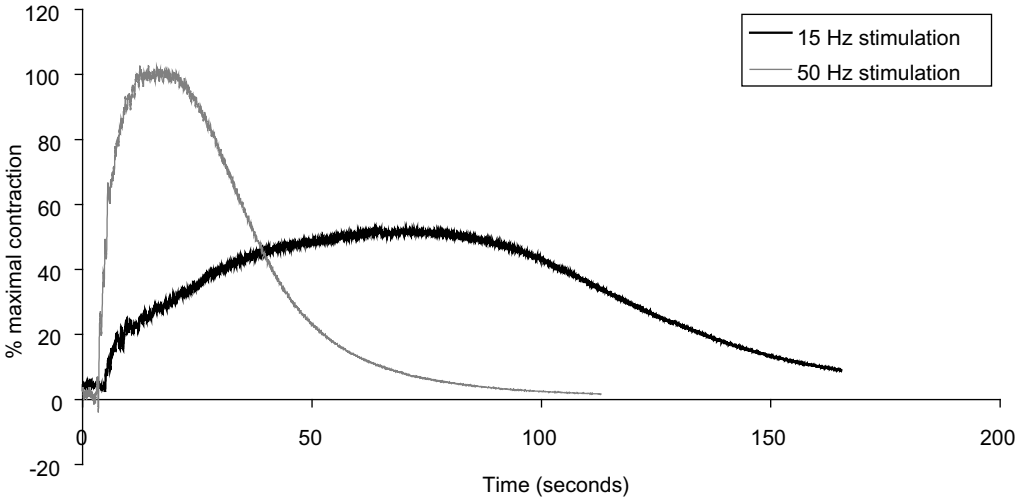

Fig. 9. Muscle pressure response in lamb carcasses stimulated at 15 and 50 Hz.

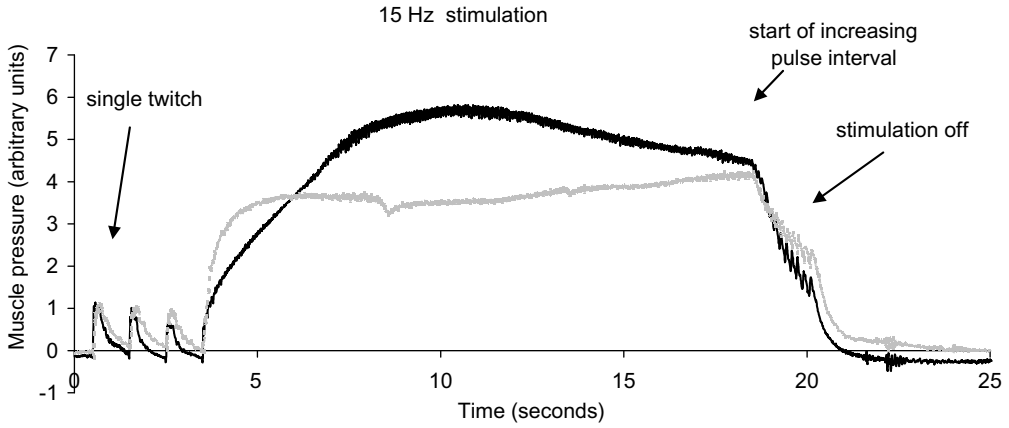

Fig. 10. Differences between lamb carcasses in muscle pressure responses to 15 Hz stimulation.

McCue, & Xenakis, 1982). The muscle pressure measurements demonstrate distinctive carcass differences in the muscle responses to identical stimulation parameters (Fig. 10). The stimulation conditions used in these experiments involved three components: first, 3 isolated individual twitch responses produced by individual 10 ms, 1 A pulses; this was followed by 12 s of 15 Hz (67 ms pulse interval) stimulation; then, 4 sets of 3 pulses, each with increasing pulse intervals of 90, 120, 150 and 180 ms. Increasing the time intervals between pulses was used to evaluate the relaxation characteristics (calcium reuptake) of the muscle. The initial rate of force development and the subsequent pattern of sustained force show clear differences in these two carcasses, and these are reflected in the pattern of relaxation in the final set of test pulses.

These measurements demonstrate that differences exist between carcasses in the contractile characteristics in response to identical stimulation conditions. In principle, measuring these contraction characteristics could provide some insight into the underlying physiological state of muscles; it would be anticipated the technique could identify differences in fibre diameter and fibre type and the physiological state of the muscle at the time of slaughter. Of more immediate practical interest, these differences probably also underlie the different magnitudes of the pH responses seen in response to electrical stimulation and need to be controlled if electrical stimulation is to be a reasonably precise technology for managing and enhancing meat processing.

5. Practical processing considerations

LVS offers a low cost option, but has the problem that it has limited effectiveness when the stimulation is applied through the hide, particularly with sheep. For this reason, it usually means manual application of the electrodes to ensure low resistance electrode contact positions such as the neck wound, and this limits the application to low throughput operations. Also, as would be predicted from the rapid decay of responses mediated by the nervous system, standard LVS systems typically produce a submaximal pH decline during the stimulation (ΔpH) and both this and the post-stimulation rate of pH decline tends to be variable between carcasses. In contrast, HVS systems tend to result in a large ΔpH in response to the stimulation and the extent of the pH fall is relatively consistent, although often the pH after stimulation is very low (and potentially too low, resulting in over-stimulation). However, the cost of the equipment is significantly greater than LVS systems and the safety requirements for its use in a commercial environment are particularly stringent. Furthermore, the dose–response

results suggest that the electrical parameters used in conventional HVS can be supramaximal with respect to the levels needed to produce a maximal muscle contraction.

6. Effect of pre- and post-rigor pH/temperature environment on meat quality

The choice of stimulation technology will depend not only on the practical aspects of cost, safety and ease of operation but also the implications for meat quality. A significant potential risk to meat quality is excessive stimulation to produce the state of low muscle pH while the muscle temperature is still high (Hwang & Thompson, 2001; Simmons et al., 2006). This condition is, of course, well recognised in pork as the pale, soft and exudative (PSE) condition, which is caused by a spontaneously rapid pH decline in the early pre-rigor period. Perhaps not surprisingly, a large pH decline caused by vigorous electrical stimulation can induce a similar condition in beef and lamb to produce an adverse effect on tenderness, water holding capacity, colour stability and overall eating quality (Geesink, Mareko, Morton, & Bickerstaffe, 2001; Koh, Bidner, McMillin, & Hill, 1987; Ledward, Dickinson, Powell, & Shorthose, 1986; Unruh, Kastner, Kropf, Dikeman, & Hunt, 1986). Most of the effects of excess stimulation can be attributed to denaturation of muscle structural proteins and enzymes. We have been particularly interested in quantifying the temperature–pH interaction to identify the boundaries for appropriate levels of stimulation, expressed in terms of the two key variables, temperature and pH, and the best practical strategies for controlling excess stimulation.

Exposing meat to denaturing conditions produces distinctive effects on meat tenderness. It is widely recognised that high temperatures can produce contractures (heat-shortening) of a magnitude equivalent to cold contractures, but the effects on toughness are distinctly different (Tornberg, 1996): whereas cold contractures increase the initial toughness and can, if sufficiently severe, maintain a toughened state even after ageing, conditions that produce significant rigor contractures have little effect on, or even reduce, initial (at *rigor mortis*) toughness and subsequent changes in tenderness are better described as a failure to tenderise rather than actual toughening (Simmons et al., 2006). The reasons why cold- and heat-induced effects on tenderness are so distinctively different has not been effectively explained.

One possibility may relate to the extent of denaturation under conditions that produce heat-shortening. Denaturation of myosin is believed to be an important consequence that increases purge loss and colour (reflectance) as the myofibrillar lattice shrinks (Offer, 1991), and may well influence meat texture. Myosin is unusual among muscle proteins in undergoing a protective transformation as rigor bonds form when ATP is fully depleted at *rigor mortis* (Offer, 1991), but this transformation does not apply to soluble enzymes, such as those responsible for tenderisation. Therefore, an explanation based on myosin denaturation would predict that denaturing conditions would affect tenderness only during the pre-rigor period, and would not extend into the post-rigor period. However, there is now clear evidence that post-rigor exposure to denaturing conditions will affect tenderness (Thomson, Gardner, Simmons, Daly, & Thompson, in press) and this is discussed further in the section below.

A further explanation was suggested by Simmons et al. (1996), who demonstrated early activation but subsequent rapid inactivation of calpains in meat held at high temperatures during the pre-rigor period. We have since evaluated an alternative methodology to estimate calpain activity using a synthetic fluorescent mu-calpain-specific substrate, calpain substrate 1 (CalS1). The degradation of the substrate can be measured by directly adding it to a meat homogenate, which avoids the need for purification of the enzyme, and allows the presence of inhibitors, such as calpastatin, or activators to influence the total measured activity. That the measured fluorescence is specific to calpain-mediated proteolysis is supported by: inhibition by the calcium chelating agent, BAPTA; inhibition of measured proteolysis by calpastatin and calpeptin; and increased activity when the assay is run at neutral pH compared with pH 5.5 (Morgan, Daly, Johnson, Simmons, in preparation). This methodology found that the activity in meat subjected to denaturing conditions in the pre-rigor period is lower, by about 20%, compared with meat held at 15 °C (data not shown). Also, changes during the ageing period are minimal, an unexpected result since μ-calpain is reported to disappear rapidly in the post-mortem period (Boehm, Kendall, Thompson, & Goll, 1998). However, Camou et al. (2007) recently identified some residual μ-calpain activity in bovine muscle after 13 days of storage, which may suggest that the purification steps in the conventional calpain measurement procedures may result in some loss of the remaining activity. In any case, the limited changes in proteolytic activity measured by the CalS1 method do not encourage a proteolytic explanation for the effects of denaturing conditions on meat tenderness.

Some further evidence suggests that the effects of denaturing conditions on tenderness involve additional complexities. Recent work by Thomson et al. (in press), incubated samples at 15 °C through the pre-rigor period but then created post-rigor denaturing conditions by transferring the samples to 35 °C. The exposure to high temperatures was maintained for varying periods of up to 12 h before the samples were returned to non-denaturing conditions at 15 °C and aged to determine the ultimate toughness. The longer exposure times were found to cause toughening, which demonstrated that contracture is unlikely to play a role in the toughening effect and that the phenomenon is not limited to pre-rigor events.

Using a similar methodology, we increased the range of temperatures (35 °C and 40 °C) and extended the exposure time to 24 h. These results were consistent with Thomson et al. (in press): the ultimate toughness increased in proportion to the time at denaturing temperatures and this increase reached a maximum effect at 6–12 h. However, extending the exposure to denaturing temperatures out to 24 h reversed the toughening effect and resulted in a final tenderness equivalent to control levels (Fig. 11). We have found this to be a consistent effect and applies also to meat held at denaturing temperatures starting in the pre-rigor period. It appears that exposing meat to denaturing temperatures alone is not sufficient to cause tenderisation to falter; perhaps an alternative explanation is that the temperature transition, which necessarily happens before ultimate tenderness levels are attained in the shorter exposure times, have a role to play in the impaired ageing effects produced by denaturing conditions.

These results show that electrical stimulation offers benefits for accelerated tenderisation but risks creating denaturing conditions that will reverse these benefits. These risks relate to excessive pH decline, or inappropriate cooling rates. Clearly some fundamental principles relating to the effects on tenderness following exposure of pre-and post-rigor meat to denaturing conditions still need some further clarification and defining the optimum stimulation levels/chilling rates will benefit from a better understanding of this denaturation-induced toughening.

In contrast, the effects of electrical stimulation on meat colour can be understood more easily. Chiller assessment of meat colour is often a part of product specifications and can have important commercial implications. Electrical stimulation, and the resultant interaction of temperature and pH, can have significant effects on colour through effects on oxygen consumption rate. We used the

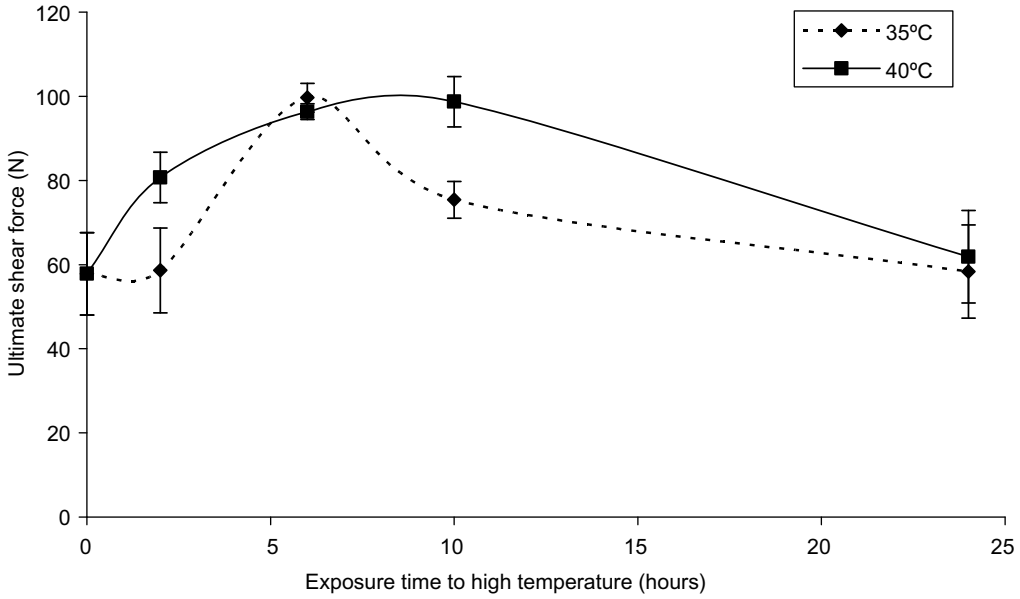

Fig. 11. Ultimate shear force in beef *m. longissimus* samples after varying periods of exposure to high temperatures immediately following *rigor mortis*. Shear force was measured using a Tenderometer on cooked samples that had been aged at 15 °C for 5 days (ultimate tenderness). Each data point represents the mean (SE) of six samples.

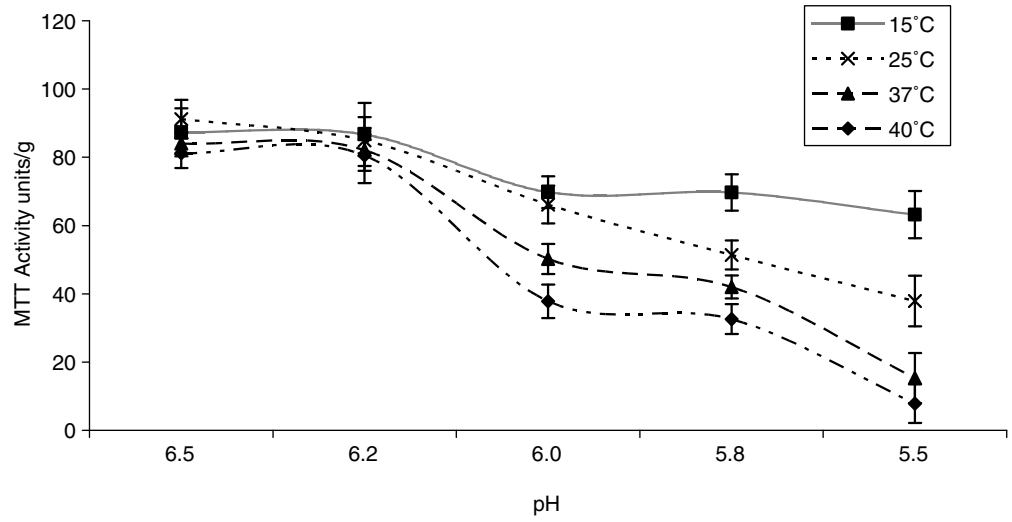

Fig. 12. MTT reducing activity in pre-rigor beef *m. longissimus* incubated at different temperatures.

reduction of a specific tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to measure the total reductive capacity of meat. Tetrazolium salts have the characteristic of being soluble and nearly colourless in the oxidised state but become coloured precipitates upon reduction, and this property has led to extensive use of this class of compounds for histological localisation of oxidoreductase activity in tissues (Stoward & Everson-Pearse, 1991). The reduction reaction is attributed to mitochondrial enzymes and electron carriers, although non-mitochondrial enzymes are also involved (Bernas & Dobrucki, 2002; Takahashi, Abe, Gotoh, & Fukuuchi, 2002). Primarily, the reduction of tetrazolium salts is an indicator of cellular 'redox activity' and, by choosing a salt with an appropriate redox potential, activity in specific steps in the mitochondrial electron chain can be assessed. MTT is potentially particularly useful because it is reduced not only mitochondrially (subsequent to cytochrome c binding to cytochrome oxidase) but also through extra-mitochondrial electron carriers, probably cytochrome b5 (Berridge & Tan, 1993): This reductive capacity of meat, as measured by MTT reduction, is therefore linked to both oxygen reduction (oxygen consumption rate) and cytochrome b5 dependent metmyoglobin reductase activity.

Fig. 12 shows the decline in loss of total MTT reducing activity of a meat homogenate at four temperatures. At all temperatures, there is minimal loss in activity until around pH 6.2 but then the activity drops in a temperature and pH dependent manner. Reductive activity also continued to decline during the post-rigor period in a temperature dependent manner (data not shown).

The MTT value is expected to be closely related to the oxygen consumption rate of meat. Reduced oxygen consumption rate means better oxygenation of the bloomed surface, so MTT values should be inversely related to a^*-values or Hue values. From the same temperature dependency experiment described above, the relationship between Hue values measured from steaks at 24 h after slaughter and allowed to bloom for 3 h was an inverse linear correlation with a correlation coefficient of 0.66.

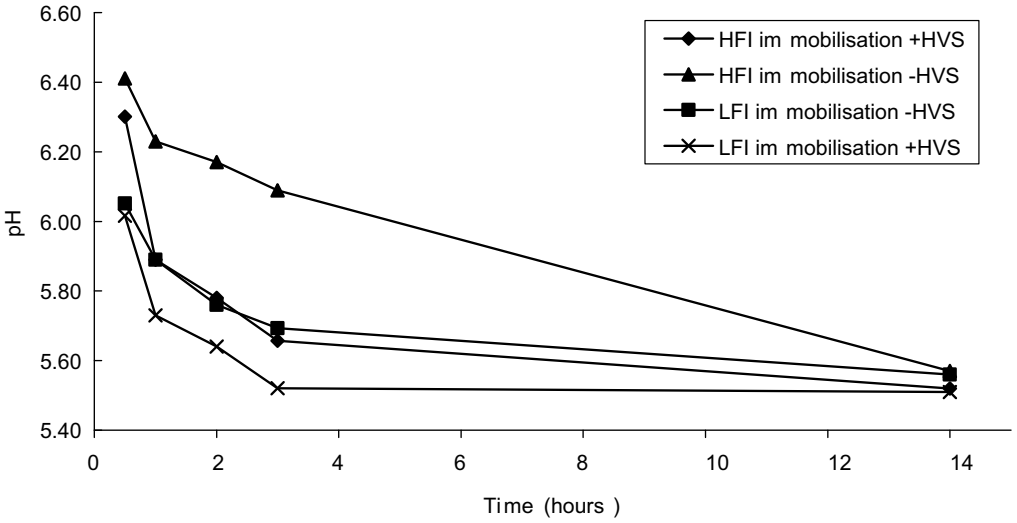

Fig. 13. The effect of either low frequency (LFI) or high frequency (HFI) immobilisation with and without subsequent high voltage stimulation on post-stimulation pH fall.

7. Commercial trials utilising different electrical inputs to the carcass

The results in Fig. 13 are from a commercial project where the requirement was to modify the pre-rigor pH/temperature environment to improve meat quality, in particular to reduce the drip loss that was evident during vacuum packed primal ageing and increase the colour stability of the product during retail display. The changes were necessitated due to evidence that the product was being overstimulated as a result of a change in the pre-slaughter stunning procedure. This was changed from captive bolt to an electrical stun, combined with post-stun electrical immobilisation to control animal movement. This process combined with the existing HVS produced too rapid a pH decline and clear signs of the PSE condition were evident particularly in the deep hind-quarter cuts.

As a solution, we replaced the conventional low frequency (14 Hz; LFI) immobilisation with a high frequency system (800 Hz; HFI) to suppress post-stun convulsive activity and allow the workers to carry out the thoracic stick and weasand location and clipping safely (Simmons et al., 2006). High frequency immobilisation is effective as a method to reduce carcass movement with minimal effects on pH decline compared with the standard low frequency systems. This difference is clearly seen in the resulting pH falls shown in Fig. 13.

The effects of modifying the rate of pH decline was compared in a 2 × 2 experimental design: LFI vs HFI, with or without HVS followed by carcass chilling generating temperatures of 12 °C after 12 h in the *m. longissimus*. Combining LFI with HVS produced the fastest pH decline and resulted in the lowest MTT reducing activity, the highest a^* value and the highest drip lost. The early tenderness values were moderate and showed poor subsequent tenderisation (Table 2). All these measurements point to excessive stimulation leading to denaturing conditions.

The lowest levels of stimulation, HFI without any further stimulation, produced a much slower pH fall and resulted in much slower tenderisation, so that the initial shear force values and those after 9 days of ageing were still higher than the other treatment groups. Further ageing will reduce the shear forces to very tender levels, but the cost of this processing specification is the long ageing times to produce optimum tenderness. However, drip losses were the lowest for this group and, while the initial colour was slightly darker, as a result of higher oxygen consumption rates, the colour stability after ageing was longer (date not shown) compared to the other groups.

The intermediate levels of stimulation (LFI+ or −HVS) produced intermediate effects on meat quality.

This example demonstrates how a processing specification can be optimised for specific meat quality outcomes by modifying electrical inputs to control the rate of pH decline relative to a chilling rate. Although not included as part of this commercial trial, chilling rate is itself a further processing variable that can be manipulated, although the influence that can be exerted by modifying temperature is much less than pH due to the thermal inertia of whole

Table 2
The effect of low and high frequency immobilisation (LFI, HFI) with or without subsequent HVS on MTT activity, a^* values, drip loss and shear force after either 2, 6 and 9 days of ageing in beef *m. longissimus* samples

	Timepoint (days)	HFI + HVS	HFI − HVS	LFI − HVS	LFI + HVS	SEM	*p*-Value
MTT	2	35.83	38.06	38.59	32.27	0.958	ns
a^*	2	19.90	18.70	20.50	21.30	0.760	ns
% Drip loss	2	0.66^{ab}	0.55^{b}	0.71^{ab}	1.03^{a}	0.060	*
	6	1.26^{b}	1.78^{b}	1.98^{ab}	2.65^{a}	0.122	***
	9	1.53^{b}	2.29^{ab}	2.13^{ab}	2.24^{a}	0.104	*
Shear force (N)	2	49.4^{b}	114.5^{c}	79.3^{a}	74.9^{ab}	0.484	***
	6	42.4^{b}	82.1^{c}	62.4^{a}	66.3^{ac}	0.304	***
	9	41.6^{b}	77.5^{a}	66.9^{a}	67.2^{a}	0.312	***

There were 20 carcasses per treatment.
Within a row, means with a different letter are significantly different ($p < 0.05$).

carcasses (this can be overcome by chilling individual cuts as part of a hot boning process).

8. Conclusions

Although now a well established processing aid, electrical stimulation technology has undergone relatively little change for many years. Meat processing is becoming increasingly complex and demanding, and the contribution of electrical stimulation needs to continue to evolve to provide greater flexibility, higher safety standards and more predictable outcomes. A far greater range of electrical parameters exist to meet these needs than are currently in use, but their development will depend on a clear understanding of the physiological principles that underlie electrically induced muscle contractions in the post-mortem carcass.

The effects of electrical stimulation on meat quality are generally recognised in a qualitative way but a fully quantitative description of the influence of electrical stimulation on quality, and one that also takes into account interactions with other processing practices still needs substantial development. We have previously identified interactions between electrical inputs, such as electrical stunning or electrical immobilisation, on the meat quality outcomes (Simmons et al., 2006) but a wider range of processing options need to be included. In particular, the benefits of electrical stimulation should be tempered by the risks of excessive stimulation and the emergence of denaturing conditions that adversely affect quality. One part of managing this risk is defining accurately the boundaries to the rate of pH decline while also considering the interactions with the chilling regimes and other processing practices. Predictive modelling techniques would appear to be ideally suited for this purpose and also will provide the meat industry with the necessary decision making support.

The second aspect of managing electrical stimulation is evolving the technology to produce a more precise and consistent processing outcome. This will undoubtedly mean recognising the differences in the response characteristics of individual carcasses, and the next generation of electrical stimulation technology will undoubtedly require some form of feedback control that allows the electrical input to be modulated to the requirement of the carcass (Simmons et al., 2006).

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References

Aston, R. (1991). *Principles of biomedical instrumentation and measurement*. New York: MacMillan Publishing Co.

Bagshaw, C. R. (1993). *Muscle contraction* (2nd ed.). London: Chapman and Hall.

Bendall, J. R. (1976). Electrical stimulation of rabbit and lamb carcasses. *Journal of the Science of Food and Agriculture, 27*, 819–826.

Bernas, T., & Dobrucki, J. (2002). Mitochondrial and nonmitochondrial reduction of MTT: Interaction of MTT with TMRE, JC-1 and NAO mitochondrial fluorescent probes. *Cytometry, 47*(4), 236–242.

Berridge, V., & Tan, A. S. (1993). Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular localization, substrate dependence and involvement of mitochondrial electron transport in MTT reduction. *Archives of Biochemistry and Biophysics, 303*(2), 474–482.

Boehm, M. L., Kendall, T. L., Thompson, V. F., & Goll, D. E. (1998). Changes in the calpains and calpastatin during the post mortem storage of bovine muscle. *Journal of Animal Science, 76*, 2415–2434.

Cairns, S. P., Flatman, J. A., & Clausen, T. (1995). Relations between extracellular [K+], membrane potential and contraction in rat soleus muscle: Modulation by the Na^+–K^+ pump. *European Journal of Physiology, 430*(6), 909–915.

Camou, J. P., Mares, S. W., Marchello, J. A., Vazquez, R., Taylor, M., Thompson, V. F., et al. (2007). *Journal of Animal Science, 85*, 3400–3414.

Chrystall, B., & Daly, C. C. (1996). Processing for meat quality. *New Zealand Journal of Animal Production, 56*, 172–175.

Chrystall, B. B., & Devine, C. E. (1978). Electrical stimulation, muscle tension and glycolysis in bovine sternomandibularis. *Meat Science, 2*(1), 48–58.

Chrystall, B. B., & Devine, C. E. (1985). Electrical stimulation: Its early development in New Zealand. In A. M. Pearson & T. R. Dutson (Eds.). *Advances in Meat Science* (Vol. 1, pp. 73–119). Westport: AVI Publishing Co.

Chrystall, B. B., & Hagyard, C. J. (1975). Electrical stimulation and lamb tenderness. *New Zealand Journal of Agricultural Research, 19*, 7–11.

Daly, C. C. (2005). Annual congress of the German Veterinary Association. In *Workshop on ritual slaughter, Berlin, Germany* (pp. 39–45).

Daly, C. C., Simmons, N. J., & Mudford, C. R. (2000). Electrical stunning and meat quality. In *Proceedings pork quality and safety summit, 11–12 July, 2000, Des Moines, Iowa, USA* (pp. 117–127).

Davey, C. L., & Gilbert, K. V. (1967). Structural changes in meat during ageing. *Journal of Food Technology, 2*, 57–59.

Davey, C. L., & Gilbert, K. V. (1975). Cold shortening capacity and beef muscle growth. *Journal of the Science of Food and Agriculture, 26*(6), 755–760.

De Luca, C. J., LeFever, R., McCue, M., & Xenakis, A. P. (1982). Control scheme governing concurrently active human motor units during voluntary contraction. *Journal of Physiology, 329*, 129–142.

Dransfield, E., Jones, R. C., & MacFie, H. H. (1980–1981). Quantifying changes in tenderness during storage in beef. *Meat Science, 5*, 131–137.

Ducastaing, A., Valin, C., Schollmeyer, J., & Cross, R. (1985). Effects of electrical stimulation on post-mortem changes in the activities of two Ca dependent neutral proteinases and their inhibitor in beef muscle. *Meat Science, 15*(4), 193–202.

Geesink, G. H., Mareko, M. H. D., Morton, J. D., & Bickerstaffe, R. (2001). Electrical stimulation – When more is less. *Meat Science, 57*(2), 145–151.

George, A. R., Bendall, J. R., & Jones, R. C. D. (1980). The tenderising effect of electrical stimulation of beef carcasses. *Meat Science, 4*(1), 51–68.

Graafhuis, A. E., Lovatt, S. J. & Devine, C. E. (1992). A predictive model for lamb tenderness. In *Proceedings of the 27th meat industry research conference, Hamilton, New Zealand, September 1992* (pp. 143–147).

Ho, C.-Y., Stromer, M. H., & Robson, R. M. (1996). Effect of electrical stimulation on postmortem titin, nebulin, desmin, and troponin-t degradation and ultrastructural changes in bovine longissimus muscle. *Journal of Animal Science, 74*, 1563–1575.

Honikel, K. O., Roncales, P., & Hamm, R. (1983). The influence of temperature on shortening and rigor onset in beef muscle. *Meat Science, 8*(3), 221–241.

Hwang, I. H., Devine, C. E., & Hopkins, D. L. (2003). The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. *Meat Science, 65*(2), 677–691.

Hwang, I. H., & Thompson, J. M. (2001). The interaction between pH and temperature decline early postmortem on the calpain system and objective tenderness in electrically stimulated beef longissimus dorsi muscle. *Meat Science, 58*(2), 167–174.

Juel, C. (1988). Muscle action potential propagation velocity changes during activity. *Muscle and Nerve, 11*(7), 714–719.

Kastner, C. L., Schwenke, J. R., Kennedy, C. J., Campbell, C. P., Kendall, J. A., & Milliken, J. (1993). Comparisons of the effect of electrical stimulation methods on postmortem pH decline in beef muscle. *Meat Science, 35*(2), 183–190.

Koh, K. C., Bidner, T. D., McMillin, K. W., & Hill, G. M. (1987). Effects of electrical stimulation and temperature on beef quality and tenderness. *Meat Science, 21*(1), 189–201.

Ledward, D. A., Dickinson, R. F., Powell, V. H., & Shorthose, W. R. (1986). The colour and colour stability of beef longissimus dorsi and semimembranosus muscles after effective electrical stimulation. *Meat Science, 16*(4), 245–265.

Marsh, B. B. (1986). The tenderizing mechanisms of electrical stimulation. In *Proceedings of IIF-IIR commission C2*, (pp. 75–81). Bristol, United Kingdom.

Martin, K. M., Hopkins, D. L., Gardner, G. E., & Thompson, J. M. (2006). Effects of stimulation on tenderness of lamb with a focus on protein degradation. *Australian Journal of Experimental Agriculture, 46*, 891–896.

McCully, K. M., & Faulkner, J. A. (1986). Characteristics of lengthening contractions associated with injury to skeletal muscle fibers. *Journal of Applied Physiology, 61*, 293–299.

Meat Technology Update (2007). Producing quality sheep meat. *Food Science Australia Meat Technology Update*, 6/07, December 2007.

Morgan, S. K., Daly, C. C., Johnson, N. J., & Simmons, N. (in preparation).

Offer, G. (1991). Modelling of the formation of pale, soft and exudative meat: Effects of chilling regime and rate and extent of glycolysis. *Meat Science, 30*(2), 157–184.

Scopes, R. K. (1971). Cold shortening in lamb. In *Meat Research Institute Memorandum No 5*. Agricultural Research Council, Meat Research Institute, Langford, Bristol.

Shaw, F. D., Eustace, I. J. & Warner, R. D. (1996). Electrical stimulation of lamb carcases. In *Proceedings of the 42nd International Congress of Meat Science and Technology* (pp. 410–411), 1–6 September, Lillehammer, Norway.

Simmons, N. J., Auld, M. M., Mudford, C. R. & Nagle, T. (1999). *Meat New Zealand Bulletin No. 55*, Meat New Zealand, Wellington, New Zealand.

Simmons, N. J., Daly, C. C., Mudford, C. R., Richards, I., Jarvis, G., & Pleiter, H. (2006). Integrated technologies to enhance meat quality – An Australasian Perspective. *Meat Science, 74*(1), 172–179.

Simmons, N. J., Gilbert, K. V., & Cairney, J. M. (1997). The effect of low voltage stimulation on pH fall and meat tenderness in lambs. In *Proceedings of the 43rd*

International Congress of Meat Science and Technology (pp. 610–611), 27 July–1 August, Auckland, New Zealand.

Simmons, N. J., Singh, K., Dobbie, P. M., & Devine, C. E. (1996). The effect of pre-rigor holding temperature on calpain and calpastatin activity and meat tenderness. In *Proceedings of the 42nd international congress of meat science and technology, 1–6 September, 1996, Lillehammer, Norway* (pp. 414–415).

Stoward, P. J., & Everson-Pearse, A. G. (1991). *Histochemistry: Theoretical and applied* (4th ed.). Edinburgh: Churchill Livingstone.

Takahashi, S., Abe, T., Gotoh, J., & Fukuuchi, Y. (2002). Substrate-dependence of reduction of MTT: A tetrazolium dye differs in cultured astroglia and neurons. *Neurochemistry International, 40*(5), 441–448.

Takahashi, G., Wang, S.-M., Lochner, J. V., & Marsh, B. B. (1987). Effects of 2-Hz and 60-Hz electrical stimulation on the microstructure of beef. *Meat Science, 19*(1), 65–76.

Thomson, K. L., Gardner, G. E., Simmons, N. J., Daly, C. C., & Thompson, J. M. (in press). Length of exposure to high post-rigor temperatures affects the tenderisation of the beef *m. longissmus* dorsi muscle. *Australian Journal of Experimental Agriculture*.

Tornberg, E. (1996). Biophysical aspects of meat tenderness. *Meat Science, 43*, S175–S191.

Unruh, J. A., Kastner, C. L., Kropf, D. H., Dikeman, M. E., & Hunt, M. C. (1986). Effects of low voltage stimulation during exsanguination on meat quality and display colour stability. *Meat Science, 18*(4), 281–293.

Wilson, J. A. (1972). *Principles of animal physiology* (2nd ed.). London: Collier Macmillan.