final report

Project Code: P.PSH. 0230

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Date published: December 2006

PUBLISHED BY
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NORTH SYDNEY NSW 2059

Super-tenderisation processes in meat

This is an MLA Donor Company funded project.

Meat & Livestock Australia and the MLA Donor Company acknowledge the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

In Australia and New Zealand, electrical stimulation has been used for more than 20 years as an integral component of both beef and lamb processing. Originally, high voltage stimulation in combination with a slow chill during the first 8 to 10 hours of slaughter was introduced into lamb processing to overcome issues with cold-shortening and generate an acceptable level of tenderness prior to freezing and shipment in a carcass form to the UK. More recently, low voltage stimulation in combination with different chilling regimes, has been introduced into both beef and lamb processing to provide greater process flexibility and optimised quality for local and export chilled markets.

Electrical stimulation is becoming an important component of sheep meat processing because it improves sheep meat eating quality and reduces variation under rapid chilling regimes used at commercial abattoirs. Sheep meat eating quality (SMEQ) standards specify that processing is optimal if meat passes through a pH by temperature window, the middle of which is18degC at pH = 6.

Optimal electrical inputs are required to accelerate post mortem glycolysis and increase the rate of pH decline post mortem. However electrical inputs are only one component of this system. The rate that carcasses are chilled will affect the decline rate of temperature by time hence the decline rate of pH by temperature post mortem. In addition a range of animal factors may affect the rate of glycolysis and the effect of electrical stimulation on the rate of glycolysis post mortem. Optimising electrical settings for sheep meat eating quality therefore requires a validation process to find settings suitable for the chilling regimes and sheep types particular to individual abattoirs and supply chains.

Current MQST research has found the benefits of low-frequency waveforms on tenderisation of sheep meat. It is hypothesised here that certain waveform that cause an eco-centric movement in the carcase during processing immediately post-slaughter causes some unexpected benefits to meat tenderness.

This research proposes a possible processing scenario for electrical stimulation of extended muscles using the following three steps :

Step 1: Transfer the carcass from Achilles tendon suspension to pelvic suspension.
Step 2: Stimulate the carcass with either the modified electrode arrangement or in the squat posture position.
Step 3: After the stimulation. Return the carcass to an Achilles tendon suspension.

The main impact of this new stimulation technique will be the additional labour required to alter the suspension procedure.

It is expected that if successful, such processing interventions will be available to processors to achieve tenderness of sheep and beef beyond normal expected tenderness of non-aged product.

Executive Summary

Electrical stimulation forms a pivotal part of processing due largely to its role in increasing the rate of tenderisation. It is generally recognised that this effect is due to the accelerated rate of pH fall during stimulation and, if high voltage stimulation is used, an accelerated rate of pH decline after the procedure. Accepting that tenderisation begins when the muscle pH reaches ultimate, then the earlier onset of rigor mortis (ultimate pH) means that the onset of proteolysis occurs earlier in the cooling curve, when the carcass temperatures are higher. Proteolysis therefore proceeds more rapidly and ageing is accelerated. However, while this can result in an acceptable process for both frozen and product intended for the local market, circumstances where reaching acceptable tenderness quickly has significant commercial advantages, other aspects of meat quality such as purge and colour stability, and even ultimate tenderness, can be adversely affected by the rapid rate of pH decline. A procedure that improves tenderness without associated loss of quality in other attributes would have significant commercial benefits

Recently, a preliminary trial was conducted to look at the effect of eccentric muscle contractions, a process of extending a muscle while it is under contraction, on tenderness in lambs. As part of the experimental protocol, different stimulation frequencies were assessed to produce different levels of contraction force: The experimental protocol used 5, 15, 50 and 150Hz, and the carcass postures involved conventional hanging, eccentric contractions and stimulation in an extended muscle state.

Eccentric contractions produced a small effect on tenderness, but this was probably not sufficient to have commercial benefit. Rather unexpectedly, a very significant improvement in tenderness was found in the 5Hz frequency group that was held in a stretched state during stimulation.

This last observation requires further development and optimisation, but an initial hurdle is to consider whether or not stimulation while muscles are in an extended state has any prospect of commercial application. This research proposes a possible processing scenario for electrical stimulation of extended muscles using 3 steps:

Step 1: Transfer the carcass from Achilles tendon suspension to pelvic suspension.
Step 2: Stimulate the carcass with either the modified electrode arrangement or in the squat posture position.
Step 3: After the stimulation. Return the carcass to an Achilles tendon suspension.

The main impact of this new stimulation technique will be the additional labour required to alter the suspension procedure. The change will require, first, a transfer to pelvic suspension and then, following stimulation, a return to Achilles suspension. Undoubtedly, some simple mechanical assistance can be designed to ease the effort involved in these steps, but additional labour units will be required. Justification for this additional cost will depend on the cost benefits that would accrue from reduced chilled storage time before freezing and benefits to product quality. Further work is required to i) quantify the benefits to tenderness of this new proposed processing technique; ii) verify through a cost benefit analysis whether the proposed additional labour is commercially viable and iii) whether any potential change of shape to cuts affects marketability or consumer perception.

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1 Background

Electrical stimulation is used extensively to accelerate the tenderisation of meat post mortem. Although in use for many years, a full agreement on the mechanism for the accelerated tenderisation has yet to be reached.

The mechanism for which there is the most convincing evidence is based on the accelerated pH decline triggered by the electrical stimulation. Accepting that tenderisation begins when the muscle pH reaches near ultimate pH, then the earlier onset of rigor mortis (ultimate pH) means that the onset of proteolysis occurs earlier in the cooling curve, when the carcass temperatures are higher. Proteolysis therefore proceeds more rapidly, and ageing is accelerated. The Accelerated Ageing and Conditioning specification developed by MIRINZ in the 1980's exaggerated this mechanism by the introduction of high voltage stimulation and the maintenance of carcasses on the cooling floor at >8deg C during the first 8-12 hours post mortem, to ensure a maximum temperature effect on the rate of proteolysis.

A second mechanism for improved tenderisation post mortem is the prevention of cold shortening. If the defining characteristic of cold shortening is an actual toughening of meat during the pre-rigor period, rather than simply slower tenderisation, then this is unlikely to be a significant issue since cold shortening even a lamb carcass would, in most cases, require sub-zero temperatures to produce the required cooling rates. Cold shortening of beef carcasses would require extraordinary chilling conditions. Low temperature-induced slowing of the aging rates is probably a better interpretation of the evidence apparent cold shortening.

A third mechanism relates to the observation of contraction nodes in myofibrils following electrical stimulation. These nodes represent areas of supercontraction in individual fibres, resulting also in areas of stretched myofibrils alongside the nodes. The studies identifying the supercontraction nodes are from American laboratories, where electrical stimulation of cattle is carried out using 50 Hz waveforms, which are usually delivered in 2 second bursts with a 1 second rest interval. The reason for the intermittent application of a 50 Hz waveform is the phenomenon of 'membrane fatigue'. At high stimulation frequencies, the muscle membranes are unable to fully repolarise between pulses and the gradual accumulation of extracellular K^+ renders the membrane unresponsive even though metabolic fatigue has not been attained and muscle pH has not declined maximally. The rest interval allows the membrane to recover and maintain responsiveness to the electrical stimulation for longer, resulting in a greater pH decline.

A second difference between a 50 Hz waveform and the more conventional 15Hz waveform used in New Zealand is a higher peak contraction force, produced as a result of greater Ca^{2+} release. The contraction nodes are likely to be caused by these high Ca^{2+} concentrations, as they do not appear to be present after stimulation at lower frequencies.

The question remains whether or not the super-contractions do actually directly affect tenderness and, at this stage, the evidence is not definitive. Furthermore, it is tempting to hypothesise that the elevated Ca^{2+} produced by high frequency stimulation may also activate the calcium-sensitive calpain enzyme systems. If so, these principles may contribute to the MQST objective of 24 hour tenderness. At this stage, there is no convincing reported evidence that the use of high frequency electrical stimulation waveforms will produce any additional benefits beyond those expected from the accelerated pH decline.

A further opportunity to accelerate proteolysis in post mortem muscle is suggested from reports of proteolytic damage to *in vivo* muscle tissue following eccentric muscle contraction. Eccentric contractions are those that require muscles to elongate while under contraction. For reasons that have not been fully defined, eccentric contractions result, within a day, in sarcolemmal damage and the appearance of intracellular muscle proteins (creatine kinase, myoglobin) in circulating blood. Evidence of loss of Z-band alignment and Z-band integrity over subsequent days suggest that proteolytic events are triggered by eccentric contraction.

The hypothesis investigated in this research is that tenderising effects of electrical stimulation can be supplemented by induced eccentric contractions. Eccentric contractions were applied to a range of different stimulation frequencies in order to generate different contraction forces through manipulation of intracellular Ca^{2+} concentrations.

2 Project Objectives

The specific objectives are:

- Evaluate the effect of eccentric restraint and novel wave forms during stimulation on ability to consistently deliver meat with a shear force value of < 7 kg F.
- Develop ES protocol for eccentric restraint and novel wave forms during stimulation to consistently deliver meat with a shear force value of < 7 kg F.

Overall, the objectives of this research is to provide a progress report on feasibility of applying unique waveforms using existing MQST technologies in processing operations to produce “super tender” meat.

3 Methodology

3.1 Evaluate the effect of eccentric restraint and novel wave forms during stimulation on ability to consistently deliver meat with a shear force value of < 7 kg F.

All work was carried out a commercial plant. Lamb carcasses were removed from the chain after dressing, approximately 30-35 minutes post slaughter. Prior to this, the animals had been stunned using a standard head-only electrical stun and Halal slaughter. During the initial work-up, the carcasses were subjected to 15 seconds of standard low voltage immobilisation (15 Hz, 10 msec pulses) although the subsequent immobilisation and stimulation rails were turned off for the duration of these trials.

For the experimental stimulation, the carcasses were removed from the commercial kill line and suspended on a purpose built stainless steel rack via a hook through the pelvis. This suspension method effectively put the hind-quarters of the carcasses into a squat-like posture.

The carcasses were stimulated via electrodes attached to the neck and pelvis. pH measurements were made immediately before stimulation and again immediately following stimulation. Thereafter, the carcasses were returned to the commercial kill floor. The day following slaughter, a sample of loin was removed from each carcass and held on ice until they were cooked for shear force measurements. The samples were cooked to an internal temperature of 75°C, cooled on ice and the shear force measured using a tenderometer.

All stimulation waveforms were based on 3 Amp (peak) unipolar pulses. Three stimulation frequencies were used to generate different contraction forces and intracellular Ca++: 5 Hz, 10 msec pulses; 50Hz, 5 msec pulses; 150Hz, 3.3 msec pulses (50% duty cycle). In addition, the waveforms were applied either continuously (S1) or intermittently using bursts of 2 seconds on and 1 second off (S2). Sixty seconds of stimulation was used throughout.

Eccentric contractions were produced during stimulation using two methods; by manually pulling the hind legs downward and releasing, at a rate of approximately 1/second (E1). During intermittent stimulation, 2 extensions were possible during each burst of stimulation. The second treatment consisted of extending the legs before the onset of the stimulation and holding the legs in this extended position for the duration of the stimulation procedure (E2).

A control stimulation treatment was also used – this consisted of stimulating the carcasses without any eccentric contraction using a standard 15Hz, 10 msec pulse curent while the carcasses were suspended via a hook through the achilles tendon (as they would be during typical commercial stimulation).

Therefore, in summary for each of the 3 stimulation frequencies, there were 4 combinations of treatment; E1 x S1, E1 x S2, E2 x S1, E2 X S2, this produced 12 treatments and a control group. A total of 260 carcasses were used providing 20 per treatment.

3.2 Develop ES protocol for eccentric restraint and novel wave forms during stimulation to consistently deliver meat with a shear force value of < 7 kg F.

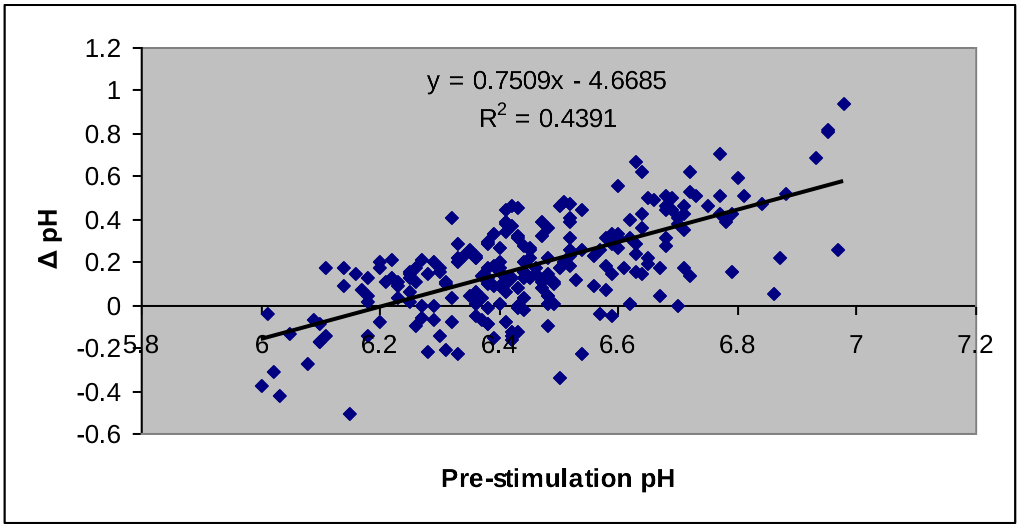
A preliminary trial investigated the effect of eccentric muscle contractions, a process of extending a muscle while it is under contraction, on tenderness in lambs. As part of the experimental protocol, different stimulation frequencies were assessed to produce different levels of contraction force: The experimental protocol used 5, 15, 50 and 150Hz, and the carcass postures involved conventional hanging, eccentric contractions and stimulation in an extended muscle state.

4 Results and Discussion

4.1 Evaluate the effect of eccentric restraint and novel wave forms during stimulation on ability to consistently deliver meat with a shear force value of < 7 kg F.

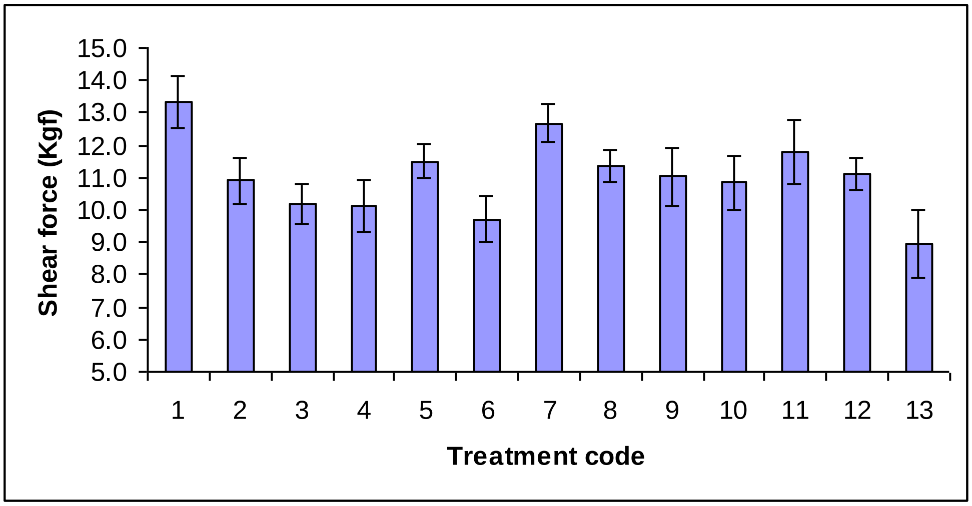
The pH decline during stimulation (Δ pH) ranged from nil to 0.31 pH units with an average Δ pH across treatments of 0.18. Across all treatments, there was a significant relationship between the pre-stimulation pH and the Δ pH (r^2 0.42 - Figure 1).

Figure 1. Relationship between the pre-stimulation pH and Δ pH



However, there was no relationship of either Δ pH or the pH post stimulation on subsequent shear force at 24 hours (data not shown).

Figure 2. The mean Kgf (± se) of lamb loins 24 hours post slaughter



Treatment code:
5Hz 1 (E1 x S1), 4 (E1 x S2), 9 (E2 x S2), 13 (E2 x S1)
50Hz – 2 (E1 x S1), 5 (E1 x S2), 7 (E2 x S2), 11 (E2 x S1)
150Hz – 3 (E1 x S1), 6 (E2 x S1), 10 (E2 x S2), 12 (E2 x S1).

Figure 2 shows the average shear force at 24 hours post mortem of all treatment groups. The treatment group with the lowest average shear force was those that had been stimulated with a continuous 5Hz current while the hind legs were held down against the body cavity. The mean shear force from this treatment group was significantly lower when compared against all the other treatment groups and the controls ($p = 0.007$). This treatment resulted in a mean shear force of 8.9 Kgf.

Furthermore, this treatment resulted in 10 samples having a shear force of ≤ 7 Kgf (Table 1).

Table 1. Effect of stimulation and eccentric contraction treatments on the number of samples with a shear force of less than or equivalent to 7 Kgf.

Hz	Eccentric contraction Hold/release (E1)	Eccentric contraction - Hold (E2)	Stimulation - Continuous (S1)	Stimulation – Intermittent (S2)	# samples Kgf ≤ 7
5	X		X		2
5	X			X	4
5		X	X		10
5		X		X	2
50	X		X		4
50	X			X	5
50		X	X		2
50		X		X	2
150	X		X		5
150	X			X	4
150		X	X		1
150		X		X	1
Control					1

4.2 Develop ES protocol for eccentric restraint and novel wave forms during stimulation to consistently deliver meat with a shear force value of < 7 kg F.

Eccentric contractions produced a small effect on tenderness, but this was probably not sufficient to have commercial benefit. Rather unexpectedly, a very significant improvement in tenderness was found in the 5Hz frequency group that was held in a stretched state during stimulation. This last observation requires further development and optimisation, but an initial hurdle is to consider whether or not stimulation while muscles are in an extended state has any prospect of commercial application.

Possible scenario for electrical stimulation of extended muscles:

The original expectation was that eccentric contractions would produce the best tenderising effect, and this would require that the muscle be stretched and released repeatedly during the stimulation period. The observation that stimulation in the extended state produced a better result actually simplifies the procedure.

Two procedures could be considered. In both, the carcass would need to be hung by pelvic suspension, as shown in Figure 3.

Figure 3: Lamb carcass hung by pelvic suspension.

Pelvic suspension has been developed in both lamb and beef as a technique to improve tenderisation by forcing muscles to enter into rigor in the stretched state. In the current procedure, the intension is only to maintain the stretched condition during stimulation, rather than throughout the pre-rigor period.

The advantage to this is that the carcass shape is not altered relative to the industry norm, and complications relating to the arrangement of carcasses in the chiller caused by the altered posture are avoided.

This research proposes a possible processing scenario for electrical stimulation of extended muscles using 3 steps :

<u>Step 1</u>: Transfer the carcass from Achilles tendon suspension to pelvic suspension.

Once in this configuration, two options are possible:

1. Position the earth potential rubbing bar above the hind legs and use the rubbing bar to force the hind legs downward, to extend the *M. longissimus dorsi* muscle, as well as the majority of the leg muscles. The effect of this position on the earth rail would be to cause the carcass to rotate ventrally about the pelvic suspension, and would therefore require that the live electrode be positioned in the ventral plane to counter this rotation.
2. Probably a better alternative would be to adopt the ‘squat posture’ handing technique. While this also requires pelvic suspension, the legs are also folded back and tucked

under a horizontal bar attached to the stem of the pelvic hook just above the carcass. This modification means that there would be no need to reposition the rubbing bars and, because the force generated by the contraction during stimulating would be countered by the horizontal bar maintaining the squat posture, there would be little rotational force generated by the carcass.

Step 2: Stimulate the carcass with either the modified electrode arrangement or in the squat posture position.

Step 3: After the stimulation. Return the carcass to an Achilles tendon suspension.

The squat posture hanging procedure was developed at MIRINZ before the advent of electrical stimulation as a mechanism for accelerating tenderness but did not prove to be a commercial success because of the change in the shape of the leg. In this application of the technique, the normal shape of the leg will be resumed once the carcass reverts back to the Achilles tendon suspension.

The main impact of this new stimulation technique will be the additional labour required to alter the suspension procedure. The change will require, first, a transfer to pelvic suspension and then, following stimulation, a return to Achilles suspension. Undoubtedly, some simple mechanical assistance can be designed to ease the effort involved in these steps, but additional labour units will be required. Justification for this additional cost will depend on the cost benefits that would accrue from reduced chilled storage time before freezing and benefits to product quality.

The cost benefit analysis will be carried out as part of the further development of these procedures during the next proposed phase of R&D (proposed for 2006-07).

5 Commercial Impacts of this Research

Electrical stimulation forms a pivotal part of processing due largely to its role in increasing the rate of tenderisation. It is generally recognised that this effect is due to the accelerated rate of pH fall during stimulation and, if high voltage stimulation is used, an accelerated rate of pH decline after the procedure. Accepting that tenderisation begins when the muscle pH reaches ultimate, then the earlier onset of rigor mortis (ultimate pH) means that the onset of proteolysis occurs earlier in the cooling curve, when the carcass temperatures are higher. Proteolysis therefore proceeds more rapidly and ageing is accelerated. However, while this can result in an acceptable process for both frozen and product intended for the local market, circumstances where reaching acceptable tenderness quickly has significant commercial advantages, other aspects of meat quality such as purge and colour stability, and even ultimate tenderness, can be adversely affected by the rapid rate of pH decline. A procedure that improves tenderness without associated loss of quality in other attributes would have significant commercial benefits

The main impact of this new stimulation technique will be the additional labour required to alter the suspension procedure. The change will require, first, a transfer to pelvic suspension and then, following stimulation, a return to Achilles suspension. Undoubtedly, some simple mechanical assistance can be designed to ease the effort involved in these steps, but additional labour units will be required. Justification for this additional cost will depend on the cost benefits that would accrue from reduced chilled storage time before freezing and benefits to product quality.

The cost benefit analysis will be carried out as part of the further development of these procedures during the next proposed phase of R&D (proposed for 2006-07).

6 Conclusions

The objective of these experiments was to evaluate the effect of eccentric contractions and the possible interactions with stimulation frequencies. Eccentric contractions are known to contribute to in vivo muscle damage during exercise, and the expectations were that the 50 Hz contractions were most likely to produce muscle damage because this frequency produces the maximum contraction forces. Unexpectedly, 5Hz produced the greatest effect on accelerated tenderisation. Furthermore, a significant effect was found in the experimental treatment where the muscles were held in an extended position: this treatment does not produce eccentric contractions, since the muscle is not being forced to extend during force generation. The treatment instead produces a partially isometic contraction.

At this stage, it is difficult to propose a possible mechanism for this 5 Hz effect. It is unlikely that supercontraction nodes are responsible, since the characteristic nodes could not be found following stimulation at this frequency, and also because the leg restraint will have prevented any significant muscle shortening in the lumbar LD.

It is quite possible that proteolytic events are involved, and the stretched state of the muscle during periods of high intracellular Ca2+ may have a role to play. In the extended state, the thick and thin filaments are minimally overlapped, and the titin molecule is in a stretched state. It is possible that protease binding sites are exposed under conditions where the muscle is extended, and this leads to accelerated proteolytic degradation.

In summary, these preliminary data show that a significant tenderising effect can be generated using a 5Hz stimulation current applied while the lamb carcass is in a squat posture position with the hind legs clamped into the body. The reasons for the accelerated proteolysis are not apparent at this stage, although future work should investigate the putative mechanisms to enable further exploitation of this technique.

It is expected that if successful, such processing interventions will be available to processors to achieve tenderness of sheep and beef beyond normal expected tenderness of non-aged product.

7 Recommendations

The conventional view of electrical stimulation is that the accelerated tenderness can be attributed to the faster pH decline. The results of the eccentric contraction experiment (muscles being stretched whilst being stimulated) in June 06, found a very significant improvement in tenderness above and beyond the known effect of faster pH decline ('enhanced' tenderness effect). The main benefit was found using a low frequency (5Hz) while the muscle was held in a stretched state.

There is now clear evidence that the use of high frequency stunning and immobilisation waveforms have a measurable impact on meat quality attributes. These are evident in meat colour, colour stability and meat texture, independent of effects on tenderness. There has as yet been no investigation of these mechanisms or how they could be exploited more effectively.

Further research and development is required to quantify the benefits of the proposed 3 step tenderisation processes.

The main impact of this new stimulation technique will be the additional labour required to alter the suspension procedure. The change will require, first, a transfer to pelvic suspension and then, following stimulation, a return to Achilles suspension. Undoubtedly, some simple mechanical assistance can be designed to ease the effort involved in these steps, but additional labour units will be required. Justification for this additional cost will depend on the cost benefits that would accrue from reduced chilled storage time before freezing and benefits to product quality.

Objectives for 2006-7

1. Further evaluate the relationship between frequency and carcass posture on the 'enhanced' tenderness effect.
2. Use the calpain and susceptibility assays to evaluate potential mechanisms ' associated with the 'enhanced' tenderness effect
3. Quantify the impact of high frequency electrical inputs on colour stability, water binding capacity and textural attributes.
4. Define the mechanisms associated with high frequency electrical inputs on meat quality attributes.

The cost benefit analysis will be carried out as part of the further development of these procedures during the next phase of R&D (proposed for 2006-07).

Finally research may be required to determine whether any potential change to shape of cuts affects marketability or consumer reaction.