final report

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Super-Tenderisation Processes

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Executive summary

Previous work, as reported in earlier milestones, has failed to identify a waveform that could generate a consistent super tender effect (defined as acceptably tender within 24 hours of slaughter). Over 1000 carcasses have been used in previous experiments with stimulation waveforms consisting of a range of frequencies, hybrid waveforms and frequencies with differing duty cycles. While several have generated some super tenderization effect, we have been able to consistently generate the response across a number of treatment days.

The hope was that we would be able to identify a relationship between an effective response to a super-tenderisation waveform and characteristics of muscle contraction measured by the muscle pressure technique. Once a mechanism had been identified, the expectation was that it would be easier to identify appropriate waveforms to exploit the specific muscle response characteristics. Unfortunately, a clear relationship was not found. However, more recently, identification of a new series of waveform possibilities became evident during discussions with Ian Richards of MLA. Preliminary trials with a sample of these demonstrated some interesting results (as reported in milestone 2) and these have been further developed and trialed recently. This waveform approach offers a large number of options and these will now be systematically screened using large sample numbers and evaluated for the super tenderization effect.

The next phase will involve screening potential waveforms based on the amplitude frequency modulated waveform approach in beef and evaluate on shear force outcomes.

Contents

Background

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 is18'C 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. In the current research, specific programmed waveforms to achieve this eco-centric event during processing will be studied. 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.

The accelerated tenderness associated with electrical stimulation is attributed to the faster pH decline; the earlier onset of rigor mortis means that proteolytic events begin sooner while the carcass is at a higher temperature. The super-tenderisation project is exploring additional mechanisms that might be exploited to accelerate tenderisation. The benefits of using hybrid waveforms for electrical stunning, immobilisation and stimulation have not been fully explored, but the results of the low frequencies on extended muscles and benefits of high frequencies on other quality attributes point towards new opportunities for using complex waveforms to manipulate muscle function and meat quality. Using this approach, it may be possible to localise the stimulation response to the loin by applying independent circuits to the legs and shoulders, so selecting 'beat' frequencies based upon the area of the carcass that requires preferential stimulation.

Project Outline

The following are the milestones:

Milestone	Milestone Description
1	Assess existing tenderisation waveforms on variable muscle response characteristics on sheep carcasses and identify key contraction characteristics that contribute to accelerated tenderisation. Quantify the nature of the accelerated tenderisation using the calpain and susceptibility assay. Interim report to MLA & MWNZ.
2	Report on the effects of tenderisation waveforms in beef cattle and their muscle pressure responses.
3	Report on strategies to modulate tenderisation waveforms to produce optimum tenderisation responses in sheep and beef.
4	Report on the effect of tenderisation waveforms on meat colour, colour stability and purge losses in beef and lamb. Compare with fast rigor conditions using the myofibrillar density and MTT assays.
5	i) Develop an industry bulletin identifying the commercial benefits of utilising a supertenderisation waveform in both beef and sheep. This document will also include details of the electronic technology required to generate these waveforms and any necessary modifications to existing stimulation rails. ii) Develop documentation and guidelines including, specifications, data sheets & materials to hand over to commercialiser(s) on operation of commercial supertenderisation technology. Transfer documents to commercialiser(s).

Project Objectives

At the completion of the Project, M&WNZ will have completed the following to MLA's satisfaction:

- Develop an industry bulletin identifying the commercial benefits of utilising a super tenderisation waveform in both beef and sheep.
- Develop documentation and guidelines including specification, data sheets and material to handover to commercialiser(s).

Experimental work

Pulse width trials: The pulse width treatments were applied on dressed ½ carcasses via the smart stimulation rail (20-25 minutes post slaughter) using the mid voltage electronics (1 Amp at 300V) with the controller programmed to deliver the 3 pulse width treatments (0.1, 0.5, 1.0 msec) at a frequency of 15Hz and the control group using 10 msec pulses at 15Hz.

AFM Waveforms: The AFM waveforms were applied in the bleeding area (hide-on, uneviscerated carcasses). The current controlled system could not be used with multiple carcasses in the smart stimulation tunnel, so a constant voltage waveform that modulated both pulse frequency and pulse width was used instead (FM modulation). The AFM waveforms were modulated with a maximum current of 2 Amps while the FM waveforms generated 1 Amp at 300V.

The controllers for both the experimental stimulation unit that was used in the bleeding area and the smart stimulation unit were programmed with the various waveforms prior to the start of the trial. Depending upon stock availability, either 2 or 3 waveforms were tested in any one day and compared to a control group, the standard 300V, 15Hz stimulation applied using the smart stim rail. The pH fall following stimulation was measured at 1.5 (pH1) and 2.5 hours (pH2) post mortem while the carcasses were in the chillers.

The following day, a sample of striploin was removed during the usual boning operations, and returned to the Carne Technologies laboratory. On arrival, the ultimate pH of each sample was measured and it was then cut into two equal pieces, one was allocated to bloomed colour evaluation and the other for shear force evaluation. A further small sample (less than 5g) was removed from the shear force samples, this was used to measure the water binding capacity.

The colour samples were placed on a polystyrene tray and overwrapped with a standard oxygen permeable retail overwrapping film. The samples were allowed to 'bloom' for 3 hours at 2 deg C and then the colour was measured using a Minolta colour meter (CR 400). The lightness, (L*), redness (a*) and yellowness (b*) values were collected and these were also converted into hue angle (3 dimensional colour) and chroma (colour intensity).

The water-binding capacity was determined by calculating the meat area and the liquid area after pressing 500 mg of sample on a filter paper sandwiched between two Perspex plates and pressed at a standard pressure for 1 minute. Photos were taken and the areas were measured using digitising software.

The shear force samples were cooked in accordance with the protocols for shear force evaluation. After overnight cooling of the cooked samples, the shear force was measured using the prototype G2 tenderometer developed under contract to MWNZ and MLA.

The data were analysed using a general linear model and differences between treatments were derived from one-way ANOVA (Minitab 14 – 2006). As with previous trials, the so called 'super tender' effect was defined as samples with a shear force equivalent to or less than 8 kgf. However, previous trials have used the original pneumatic tenderometer where the shear force scales tend to be higher. In this trial, all samples were evaluated using the prototype G2 tenderometer and therefore the super tender cut off was re-defined using the previously derived conversion equation which allows G1 shear force values to be converted to G2. This effectively generated a G2 super tender cut off of equivalent to or less than 5.7 kgf.

Results & Discussion

Milestone 1 & 2

Trials evaluating potential super tenderisation waveforms involved application of the waveforms during the bleeding area (hide-on, uneviscerated carcasses) and post dressing using the existing stimulation tunnel (half carcasses via a rubbing rail – 20-25 minutes post slaughter).

The waveforms that have been tested varied both pulse frequency and pulse width and more recently, a waveform that modulates both amplitude and frequency was trialled. The procedures for these trials involved evaluating the effect of the waveforms on the post-stimulation pH response and on shear force (and other quality attributes).

The tested waveforms included increasing stimulation frequencies, which has the effect of increasing the peak force of contraction. To avoid membrane fatigue at these high frequencies, the waveforms were tested as on/off bursts to allow the muscles periods of recovery.

Doublet waveforms are based on two pulses in rapid succession. These waveforms have all been shown in the physiology literature to generate more persistent muscle contraction as the muscle fatigues.

Simulation was carried out either pre-dressing, during bleeding, or post dressing on half carcasses. In both cases, the stimulation voltage was 300 Volt, which ensured a delivery of at least 1 Amp. Pre-dressing stimulation was delivered via electrodes attached manually to the nose and through the overhead rail, for a duration of 60 seconds. Post-dressing stimulation employed rubbing electrodes and lasted 90 seconds.

For each waveform, 10-20 carcasses were tested. The carcasses were chilled according to the normal process and a portion of the sirloin collected in the boning room. The samples were held on ice and cooked at 24 hours after slaughter. The objective was to identify waveforms that produced an increase in the number of samples with shearforces <8 kgf at 24 hours. Each trial included control carcasses that received high voltage stimulation (10 msec pulses, 1134V peak) for 90 seconds.

1: Pre-dressing stimulation.
Six different waveforms were evaluated in the bleed area. The waveforms shown in Figure 1 did not generate a significant change in the shear forces of the striploin at 24 hours post slaughter (the target is ≤ 8kgf).

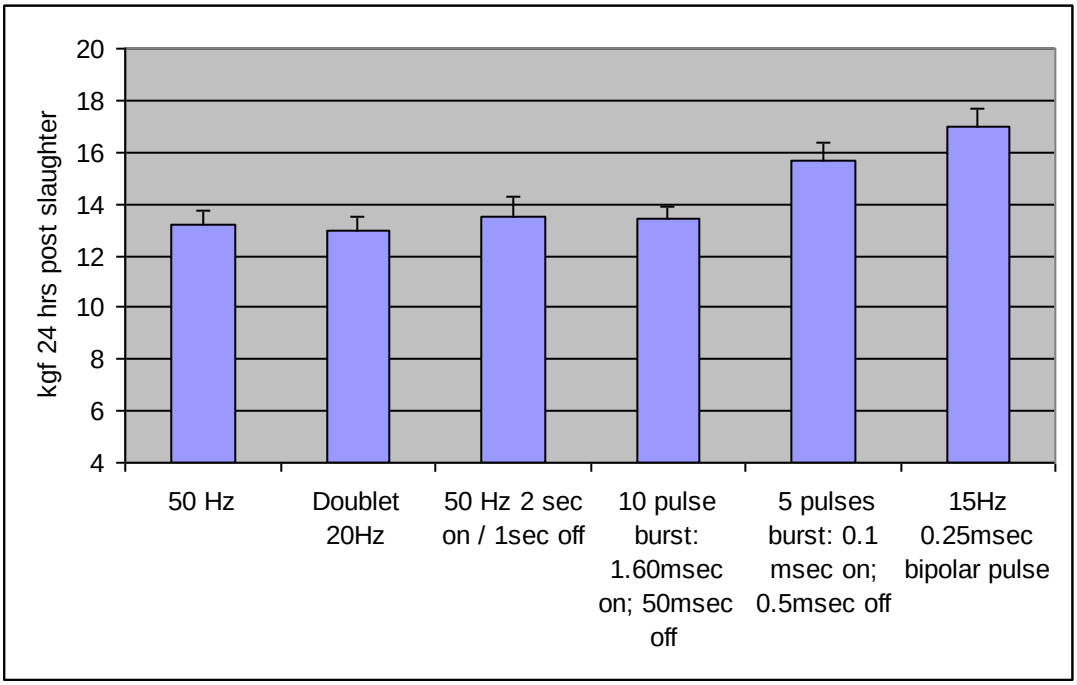


Figure 1: Potential super-tenderisation waveforms applied in the bleeding area.

2. Post dressing stimulation
A range of pulse widths and pulse frequencies were tested in carcasses following dressing (Figure 2).

The effect of varying the pulse width in a conventional 15Hz stimulation waveform produced some unexpected results. First, the pH decline was not significantly affected by pulse width, even when the pulse width was reduced to 0.5 msec.

Second, the tenderness results found a significant decrease in mean tenderness, and increase in the number of samples <8 kgf (8/20; 40%), at 1 msec pulse width. This compares with 15,15 and 5% for pulse widths of 0.5, 5 and 10 msec, respectively.

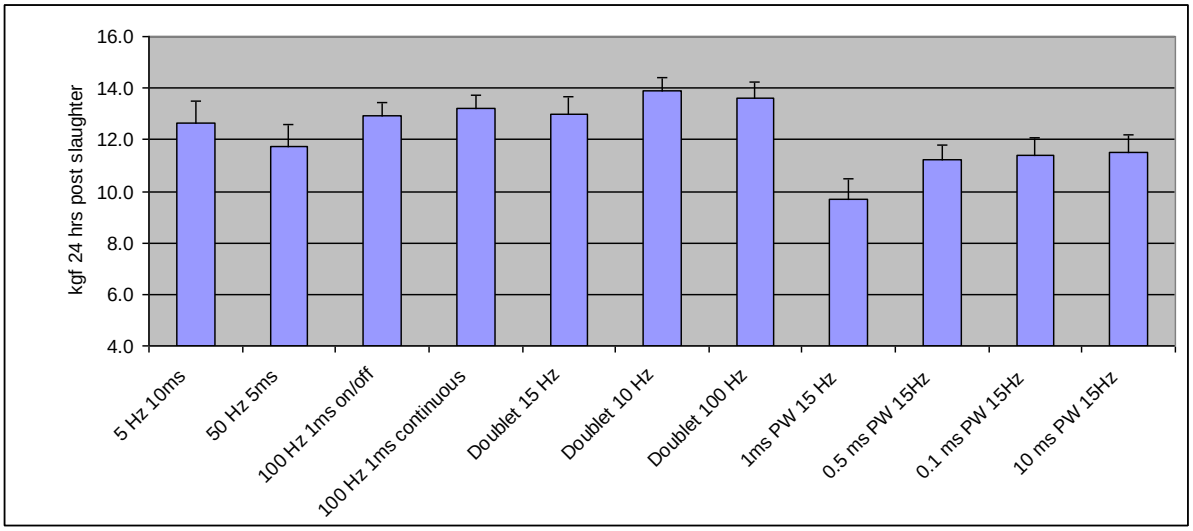


Figure 2: potential super-tenderisation waveforms applied post-dressing using the existing stimulation tunnel.

3. Amplitude and frequency modulated (AFM) waveform.

A specialised waveform that combined both amplitude and frequency modulation (Figure 3) was tested. The waveform was delivered as either repeated 30 or 200 msec pulses, with a peak amplitude of either 1 Amp or 200 mA. This waveform was delivered pre-dressing.

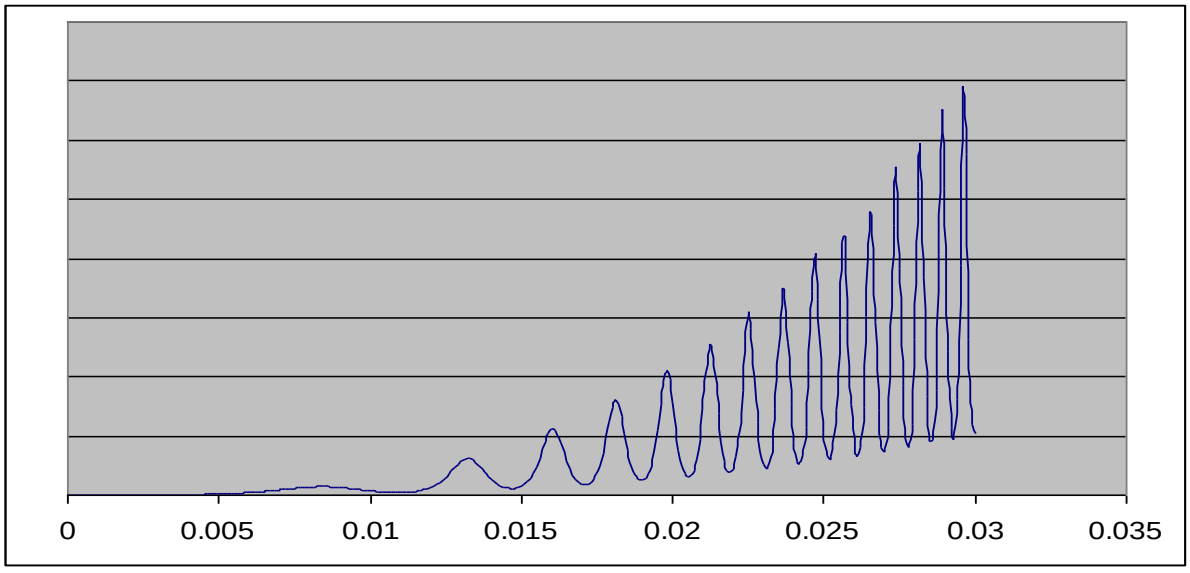


Figure 3: AFM waveform, using a 30 msec pulse.

The AFM waveform at 1 Amp produced a vigorous pH decline: at 3 hours post dressing, the pH was 5.74 and 5.85 for the 30 and 300 msec pulses, which is equivalent to an average pH of 5.8 following high voltage stimulation. When the amplitude of the AFM waveform was reduced to 200 mA, the pH decline at 3 hours was increase to 6.03 and 6.11 (high voltage control was 5.84).

The effects on tenderness for the 30 msec pulses, high amplitude pulses were equivalent to the high voltage control tenderness results; 24 hours shear force was 14.2 compared with 14.3 in the controls. In contrast, the 300 msec pulses resulted in high shear force at 24 hours (18.6). At the lower amplitude, the 30msec pulses did not quite achieve the tenderness of the high voltage controls (14.7 vs 12.6 respectively) whereas the 30msec pulses remained tough (19.4).

Milestones 3, 4 & 5

Trials evaluating potential super tenderisation waveforms were carried out in beef at Auckland Meat Processors. Two approaches have shown some promise during past trials; 1. effect of varying pulse widths and 2. a frequency modulated waveform. While these results did not suggest that pulse width modifications alone are likely to generate consistent super-tenderisation effects, understanding the effects of pulse width will help refine the waveforms, particularly those that have recently been identified that use an amplitude and frequency modulated waveform (AFM & FM).

Therefore, this report consists of two parts; the first was to build on the preliminary pulse width data that was reported in milestone 2; and the second was to start screening the AFM waveform options.

Pulse width trials

An earlier milestone identified some unexpected results using a range of different pulse widths delivered at the traditional 15Hz stimulation frequency. This work was repeated using between 60-80 carcasses per treatment to evaluate the effect of varying pulse widths on the pH at various times post mortem.

The results were broadly similar to those reported previously; the pH fall following a 15Hz stimulation using 0.1 msec pulse widths was significantly less compared to pulse widths of 0.5, 1 and 10 msec but there were no differences between these last three (Table 1).

Loin samples from a total of 60 samples per treatment were selected during the course of these trials and the shear force was measured at 24 to 26 hours post mortem. The results in Table 1 show that there was no significant effect of pulse width on the average shear force at 24 hours post mortem although the highest mean shear force values were from the 10 msec control and 0.1 msec treatment groups (Table 1). Looking at these data for evidence of the super-tender effect found that, consistent with previous reports, the 1 msec pulse duration produced the greatest proportion of super tender samples (27%). By comparison, the control group, using a 10 msec pulse duration, generated only 9% of samples in this category.

Table 1. Effect of stimulation applied to dressed carcasses (via smart stim rail) using varying pulse widths at 15Hz

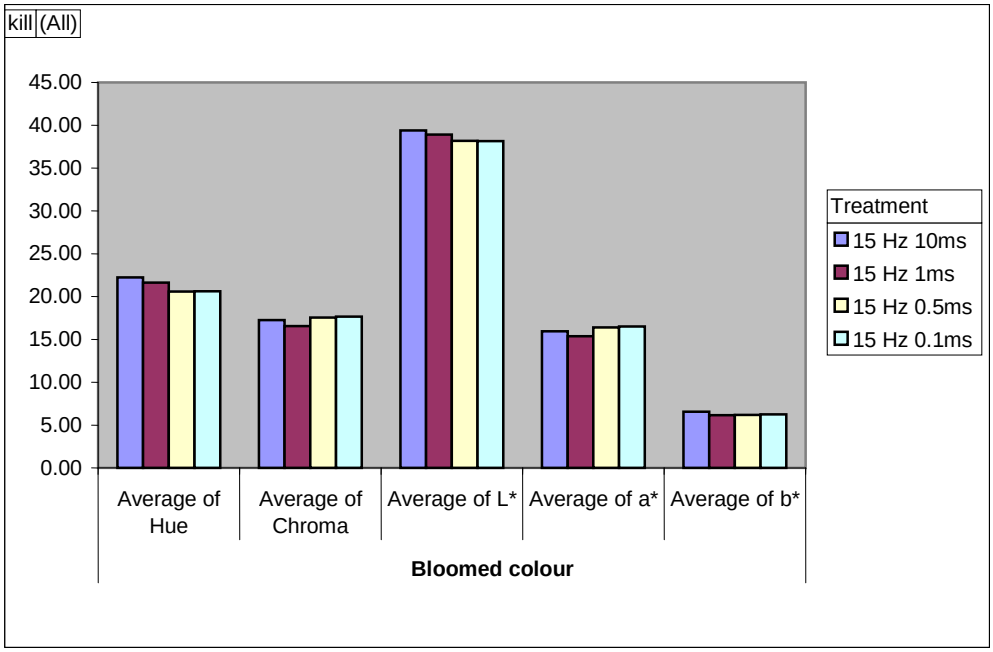
Treatment	pH1	pH2	Shear force (1 day post mortem	% in the 'super tender' category
15 Hz/0.1 msec	6.17[a] (0.01)	5.91[a] (0.02)	6.51 (0.29)	20.0
15 Hz/0.5 msec	6.10[b](0.01)	5.84[b] (0.02)	6.42 (0.20)	15.0
15 Hz/1.0 msec	6.11 [b] (0.01)	5.84[b] (0.02)	6.44 (0.23)	27.1
*15 Hz/10.0msec	6.10 [b] (0.01)	5.84 (0.01)[b]	6.72 (0.14)	9.1
Significance	P<0.01	P<0.01	NS	N/A

* Control group

The effect of pulse width on bloomed colour is shown in Figure 4. To summarise these data, the 0.1 and 0.5 msec pulse width treatments resulted in a slightly better bloomed colour; the samples were a stronger, slightly darker red with less of a brown component when compared to both the

1.0 and 10 msec pulse width treatments. Despite these differences, all treatments generated a bloomed colour that would be regarded as acceptable if assessed on retail display by consumers.

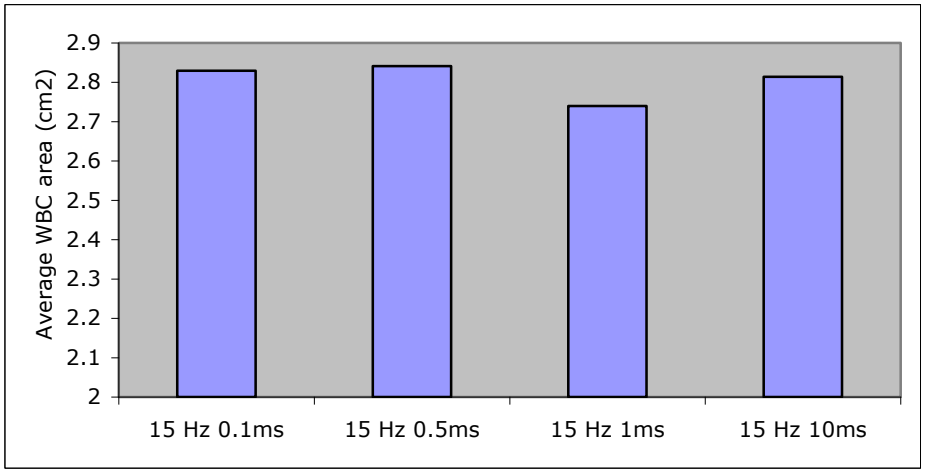
Figure 4. The effect of varying stimulation pulse widths on bloomed colour



There were no effects of pulse width on the amount of expressible water (water binding capacity) (Figure 5) although the average value for the 1 msec pulse width treatment was slightly less than the other three treatments.

Typically, the water binding capacity of meat declines with faster rates of rigor onset; the more the myofibrillar proteins are denatured during the pre-rigor period, the less they are able to maintain intramuscular fluid during post-rigor storage and thus the higher is their expressible fluid. For these data, the higher water binding capacity is produced by the 1.0 msec treatment although these differences are not significant (Figure 5). This is in contrast to the pre-rigor pH falls, where the slowest rate of decline was measured in the 0.1 msec samples. However, the differences in both pH decline and subsequent WBC between treatments are relatively small and therefore do not have any commercial value.

Figure 5. The effect of varying pulse widths on water binding capacity



Modulated waveforms

Three separate trials of each modulated waveform, involving 15-20 carcasses per treatment, were carried out. The AFM waveforms, delivered during bleeding, were based on a waveform described in the previous milestone. This waveform was generated at 3 different frequencies, described using arbitrarily units as 9.766, 78.125 and 156.25 and which represent a 1/8 and 1/16 change in the baseline frequency.

Two separate FM modulated waveforms were assessed. Waveform one involved only a modulated pulse width, which varied between 0.2 and 20 msec in 5 decreasing and 5 increasing steps, at a continuous 15 Hz over a time base of 2.6 seconds. The second waveform varied both pulse width and pulse frequency, varying from 20 msec pulses at 11.5 Hz to 0.2 msec at 909 Hz, over a period of 4 seconds.

Table 2 shows the results of the different modulated waveform treatments. The AFM waveform produced a consistently lower number of super-tenderised carcasses relative to controls. While this is obviously of little commercial benefit, it still demonstrates that tenderness can be influenced by stimulation waveforms through a mechanism dissociated with the rate of pH decline.

This conclusion is reinforced by the effects of the FM waveforms, which both showed an increase in the number of super-tenderised carcasses.

Table 2. Effect of stimulation using AFM waveforms with different characteristics applied either at bleeding or on dressed ½ carcasses.

Treatment	pH1	pH2	Shear force (1 day post mortem	% in the 'super tender' category
AFM 78.125	6.25 (0.03)^{a}	6.02 (0.04)^{a}	8.94 ^{a} (0.40)	3.3
AFM 9.766	6.18 (0.03)^{bc}	5.98 (0.04)^{ab}	7.83 ^{bc} (0.26)	12.9
AFM 156.25	6.31 (0.04)^{ab}	6.14 (0.05)^{b}	7.17 ^{bc} (0.73)	11.1
FM Waveform 1	6.12 (0.02)^{c}	5.92 (0.03)^{ac}	6.27 ^{c} (0.29)	45.0
FM Waveform 2	6.23 (0.02)^{ab}	6.09 (0.03)^{ac}	6.67 ^{c} (0.32)	45.0
Control	6.12 (0.02)^{c}	5.87 (0.03)^{c}	7.85 ^{bc} (0.29)	16.3
Significance	P<0.001	P<0.001	P<0.001	N/A

Project Outcomes

<u>New Technology:</u>

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

Project Intellectual Property:
Shared between MWNZ, MLA

IP is shared between MLA and M&WNZ, on the condition that MIRINZ Inc will be acknowledged in any media release or public statement concerning the results of the MLA / M&WNZ collaborative research programme.

Commercialisation/Dissemination Strategy:
Outcomes kept confidential pending decision to continue towards a commercial technology.

Conclusion

In general, the waveforms tested showed a relationship between the rate of pH decline following stimulation and the tenderness at 24 hours, consistent with the general principle that accelerated tenderisation is largely a function of earlier onset of rigor. We were unable to demonstrate a consistent effect of increasing the stimulation frequency; these frequencies produce higher intracellular calcium and greater contraction forces, effects that have been linked with accelerated tenderisation.

Two results appear to show some uncoupling between rate of pH decline and tenderness. The first is the 1 msec pulse width using a 15 Hz stimulation frequency. This result is based on two separate trials (10 carcasses on each occasion) and, in both cases, the pH decline was equivalent for this particular pulse width compared with either shorter or longer versions and yet the shear forces were lower. This result will need further confirmation but may indicate an independent mechanism associated with pulse widths of this magnitude.

The second is the AFM waveform. In this case, the pH decline was very vigorous, particularly when a 1Amp pulse was used, and the 30msec pulses produced a shear force comparable to high voltage stimulation. However, the 300 msec pulses produced very tough meat, in spite of effective pH decline (particularly at 1Amp amplitude). While this obviously has limited commercial application, it does offer an opportunity to study tenderness (or lack of) effect independent of pH decline. Some further modulation of the waveform could identify ways of producing a commercially favourable outcome.

The results reported here are based on three replicates of each treatment and each showed a similar pattern of response to the different treatment. This suggests a reliable response, even though the effects are probably not sufficiently consistent at this stage to provide a clear commercial advantage.

The benefit of a 1 msec pulse width relative to 10 msec is particularly surprising, as was the particularly high incidence of super-tenderised carcasses with the FM waveform. These results provide encouraging new avenues for exploring super-tenderisation waveforms and will be incorporated into the new trials under MQST 08/09.

Recommendations / Commercial

Previous work, as reported in earlier milestones, has failed to identify a waveform that could generate a consistent super tender effect (defined as acceptably tender within 24 hours of slaughter). Over 1000 carcasses have been used in previous experiments with stimulation waveforms consisting of a range of frequencies, hybrid waveforms and frequencies with differing duty cycles. While several have generated some super tenderisation effect, we have been able to consistently generate the response across a number of treatment days.

The hope was that we would be able to identify a relationship between an effective response to a super-tenderisation waveform and characteristics of muscle contraction measured by the muscle pressure technique. Once a mechanism had been identified, the expectation was that it would be easier to identify appropriate waveforms to exploit the specific muscle response characteristics. Unfortunately, a clear relationship was not found. However, more recently, identification of a new series of waveform possibilities became evident during discussions with Ian Richards of MLA. Preliminary trials with a sample of these demonstrated some interesting results (as reported in milestone 2) and these have been further developed and trialed recently. This waveform approach offers a large number of options and these will now be systematically screened using large sample numbers and evaluated for the super tenderization effect.

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Acknowledgements

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