final report

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A physiological understanding of new generation post slaughter electrical technologies

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Executive summary

There is renewed interest in hot boning in the meat processing sector due to increased abattoir efficiency and increased yields. The major disadvantage of hot boning however is the potential for meat to cold shorten and become tough. New prototype meat processing equipment called the BOA being developed by Meat and Livestock Australia (MLA) can stretch the meat and can prevent rigor shortening in hot boned meat. We believe this technology should incorporate both a stretch and stimulation combined approach to optimise the rate of glycolysis and prevent rigor shortening.

The aim of this objective (Objective 5) was to determine the maximum increase in force of muscle contraction that could be obtained at 90min post mortem (likely time for hot boning) using various combinations of stretch and electrical input. As temperature is a key variable in meat processing, we also investigated how the force of contraction changes with temperature. A number of findings were observed which all have commercial implications of how a stretch and stimulation combined technology for hot boned meat can work optimally and maximise the force generation and rate of glycolysis.

1. The study showed that it is the degree of tension exerted that determines the force generated when the muscle is stimulated, not the degree of stretch of the muscle. This has commercial implications with regard to how stretch/stimulation technology is applied and what the controlled variables will be. We suggest that it will be critical to control of tension exerted rather than distance stretched. It's important to note that there is a negative effect of excessive force exertion.
2. There is optimal level of tension which will elicit a maximal force of contraction in the muscle strips at 90 mins post anoxia. The optimal tension level that achieves maximal force generation is different between muscle groups. Therefore each muscle/cut/primal will individually need to be assessed for its optimal tension level. Force generated can be further increased by manipulating voltage and pulse width.
3. The rate of decline in force generation is not similar over time between muscle types, with predominantly fast twitch muscles [type 2b (glycolytic)] such as the ST showing a greater loss in potential force generated over time. It would therefore be necessary to identify "priority' cuts that require more rapid processing.
4. Hot boning and application of stretch/stimulation technologies need to be applied as soon as possible after slaughter. The use of higher pulse widths may be a strategy to counteract for the negative effect of time on force of contraction.
5. Force amplitude decreases with decreasing temperature, however at 25-30oC the muscle strips contracted at 60-70% of the force that they would at 40oC. The incorporation of a stretch and stimulation combined technology for hot boned product is therefore best utilised in meat above 25oC for optimal force generated and therefore greater work and pH decline.
6. A decrease in muscle strip temperature changes the pattern of contractile response. Responses are lower in amplitude and show reduced ability to recover between pulses. Stimulation with high frequencies (such as the commercially used 15Hz) at lower temperatures may result in the muscle not able to complete enough contractions resulting in the muscle doing less work and therefore producing a lower glycolytic rate.

The issue arises of how the results with the muscle strips in terms of tension levels can be reflected to whole muscle/cut or primals. Tests on larger sized muscle/primals possibly in combination with the BOA need to be undertaken. This study has indicated that incorporating a stimulation component into the BOA may be a valid and highly productive option to improve meat quality. Further research and engineering with the BOA in conjunction with meat quality studies to understand the potential benefits is justified.

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Background

Electrical applications within abattoirs is now a standard processing technology, and applications include pre-slaughter stunning, immobilisation, bleeding and manipulation of pH decline. Immobilisation post slaughter is desirable in order to produce a safer work environment during shackling and legging and increasing blood recovery but is countered by the requirement to not deleteriously affect pH or induce heat shortening. Within the next 2 years medium voltage electrical stimulation machines designed to manipulate pH decline are expected to become widely used in Australian abattoirs processing lamb meat. In Western Australia, one machine has recently been installed at WAMMCO International abattoir Katanning and another is planned at V. & V. Walshes abattoir Bunbury.

The benefit of these stimulation systems is that they hasten the process of rigor mortis by causing muscles to undergo work via anaerobic glycolysis, resulting in an initial pH fall followed by a change in the rate of pH decline. The combined effect is that muscles enter rigor mortis before the muscle temperature falls to values conducive to cold shortening and toughening. Importantly, the current and pulse width settings may be altered on these machines to alter the rate of glycolysis to suit the abattoirs requirements. Increasing the current will increase the effect on glycolysis, however there is a saturation point whereby an increase past this point will not affect the glycolytic rate. Widening the pulse widths can also increase the stimulation effect.

The interaction between current and pulse widths with muscle contraction, nerve function and pH decline is not clearly understood. Therefore, the effects on tenderness remain unclear. A key component of this project will be to develop a scientific understanding of the relationships between electrical stimulation parameters, nerve and muscle responses to the applied electrical current and objective meat quality. In many circumstances, the protocol and the physiological effects are defined empirically, based on experience rather than an integrated understanding of the applied electrical parameters and the induced responses in the muscle. Our study will provide the opportunity to develop electrical current parameters that are tailored for specific applications, generate more consistent carcass responses and identify opportunities to manipulate biochemical events within nerve and muscle tissue.

Project Outline

The following are the milestones:

Milestone	Achievement criteria
1	i. Objective 1, Aim 1 – Final report on existing pH data sets from the Beef and Sheep CRC processing activities. ii. Preliminary reports on Objective 1, Aims 2 and 3. Delta pH response to electrical stimulation parameters and correlation with in-vivo contractile response to same electrical stimulation parameters
2	i. Final Reports on Objective 1, Aims 2 and 3. ii. Final report Objective 2, Aim 1 - stimulation parameters (low voltage and variable frequency and pulse width) that consistently evoke nerve-mediated and direct muscle stimulation iii. Stage 1 report on Objective 2, Aim 2. Time points experiments to establish the time frame during which the evoked contraction changes from nerve-mediated to direct stimulation.
3	i. Final report on Objective 3- Optimisation study and recommendations of use of MVES system at WAMMCO abattoir
4	i. Final report Objective 2, Aims 2 and 3 including transferred of time frame experiments to whole carcass experiments at Murdoch Universities experimental abattoir and testing in a commercial operation. ii. Stage 1 report on Objective 3, Aim 1- expression of neuromodulators and receptors in sheep and rat, fast and slow twitch fibres.
5	i. Final report Objective 4, Aims 1 and 2 including establishing the mechanism by which neuromodulators influence synaptic transmission and the excitation-contraction coupling process. ii. Final report on Objective 5 - Aim 1 - set-up nerve-muscle preparations under physiological conditions (temp, oxygen, pH) and determine twitch and tetanic responses to various high frequencies and pulse widths including increasing and decreasing ramp of stimulation frequency
6	i. Final report on Objective 5, Aim 2- High frequency stimulation experiments aimed at determining the contribution of nerve-mediated vs. direct muscle stimulation under HF conditions. ii. Final report on Objective 5, Aim 3 HF experiments under anoxic/ischaemic nerve-muscle conditions

Project Objectives

This project has 5 major objectives that aim to increase our overall understanding of the basic physiological mechanisms that influence the response of the lamb carcasses to electrical inputs. To date, we have developed an isolated nerve/muscle preparation in vitro to allow the detailed study of electrophysiological mechanisms underpinning muscle contraction in post mortem animals (initially the rat and then the sheep).

1.Establish the relationship between the initial carcass pH and response to different electrical stimulation parameters. This study will enable us to determine if the initial carcass pH can be used to optimise individual carcass stimulation parameters, and what other factors may influence this initial delta pH.
2.Establish the electrical stimulation parameters that evoke nerve versus muscle mediated stimulation in different muscle types and then understand the effects of time post mortem for these effects
3.Determine how different frequency, modulation, current and pulse width settings on a medium voltage electrical stimulation unit affect rate of pH decline, tenderness and drip loss at WAMMCO International abattoir. This study will enable final recommendations for the use of the MVES in Australia
4.Determine the action of various neuromodulators at the neuromuscular junction under physiological conditions in response to different electrical stimulation parameters. This will allow us to better understand the nerve and muscle responses to the applied electrical current and will promote our basic understanding of neuromuscular junction signalling.
5.Define the responses of muscle and nervous tissues to complex high frequency electrical waveforms that can be utilised to immobilise animals for shackling but not prematurely causing pH decline.

Experimental work

Method

Objective 1: Influence of initial carcass pH on response to differing electrical stimulation parameters

The initial rate of carcass pH decline (post slaughter) prior to stimulation is an indicator of the subsequent response to stimulation, i.e., carcasses that have a faster rate of pH decline immediately post slaughter and thus a lower initial pH at the time of stimulation will respond less to the stimulation applied. However, they also will require less stimulation to achieve the required pH-temperature window (e.g. for the export market, carcass/loin temperature needs to be 18-25°C at pH6), whilst carcasses with a higher starting pH prior to stimulation may require a larger electrical input to achieve the same endpoint.

Studies will report on 6 data sets involving highly control experimental protocols obtained from the Sheep CRC and Beef CRC and in addition commercial data will be obtained from the WAMMCO abattoir (Katanning, WA). Currently there is a study running at WAMMCO which is

investigating the effect of various stimulation parameters (i.e., altering current and pulse width) on the rate of pH decline in sheep carcasses, specifically on the tenderness, drip loss and colour stability of the end meat product. This study only examines the pH/temperature decline of carcasses subsequent to stimulation. However, knowledge of the factors influencing carcass pH prior to stimulation, and how this in turn influences the success of electrical stimulation, would be of significant value to meat producers. Between day variability is the largest source of variation and thus sampling will be structured to enable estimation of this. Further, in order not to confound the data, feedlot vs. pasture, age, genotype (eg muscling) and transport distance/lairage time will be factored into data analysis. Delta pH (representing the difference in pH measured immediately before and after electrical stimulation) in response to two different electrical stimulation parameters will be measured. Falls of 0.4 to 0.6 pH are considered effective responses.

Aims:

1. report on exisiting pH data sets from the Beef and Sheep CRC processing activities.
2.To determine the delta pH response to variation in 2 different electrical stimulation parameters (pulse width and frequency), and subsequent analysis on data sets testing for important variables that define the effectiveness of response.
3.To relate these delta pH responses to in-vitro contraction responses determined for a similar range of pulse width and frequency parameters tested in-vitro using the muscle preparation techniques described in Objective 2.

Methods:

Two different parameters varying pulse width and frequency will be tested. Currently, preliminary studies are being performed at the WAMMCO operations to determine these parameters. Resutss will be compared to control animals which will undergo no electrical stimulation. Information on animal history will be collected prior to slaughter (diet, age, genotype, transport and lairage time). Data on body composition (% fat score, % lean yield and carcass weight will also be collected). Data will be collected over multiple days (minimum n = 5, to avoid confounding data due to between day variability) and 10-20 samples will be collected for each stimulation parameter for animals from different consignments on each day. Tissue will be collected using a muscle biopsy drill immediately prior to and after electrical stimulation. The semitendinosus, semimembrinosus and longissimus dorsi muscles will be sampled. Tissue will be snap frozen in liquid nitrogen and transported to Murdoch University for accurate pH analysis using the iodoacetate method.

This project will be run parallel to in-vivo studies at Murdoch University (Aim 3), using the electrophysiology equipment to test similar combinations of frequency and pulse width parameters, measuring force of contraction. The underlying hypothesis is that those stimulation

parameters that induce the greatest delta pH will also induce the greatest amount of force generation/work in the muscle There will be on-going refinement of the two arms of the study as results are obtained.

Objective 2: Establish the electrical stimulation parameters that evoke nerve versus muscle mediated stimulation in different muscle types and then understand the effects of time post mortem for these effects.

Dependent upon the electrical parameters used and time after slaughter, the post-mortem electrical stimulation of a carcass will induce contraction of muscles via either nerve-mediated pathways or direct electrical stimulation of the muscle. A response mediated by nerve pathways is advantageous over direct electrical stimulation as it will provide a more homogenous stimulation of the carcass. This therefore will produce a more homogenous pH decline in muscle compared to electrical stimulation where some muscle bodies and fibres within muscles will have been stimulated to varying levels.

Experimental evidence has demonstrated that immediately after slaughter, low voltage stimulation induces muscular contraction exclusively by the nervous system (Morton and Newbold 1982). It is generally believed that low voltage stimulation must be applied within 5 min of slaughter since, beyond this time interval, the activity of the nervous system decays and compromises the stimulation-induced homogeneous pH decline. This has practical limitations, even for low throughput plants (Daly and Mudford 1998). A study by Daly and Mudford (1998), revealed that low voltage electrical stimulation of lamb carcasses as late as 20 min following slaughter could still elicit muscular contractions and a pH decline but via direct stimulation. The type of muscle fibre will also influence the response to nerve stimulation, with submaximal inputs being more likely to stimulate large glycolytic fibres, contributing further to heterogenous pH declines

Two of the fundamental aspects identified with post-mortem muscular responsiveness to nerve stimulation that were identified from the initial trial (Daly and Mudford 1998) are the loss of nervous activity with time, and a lowered threshold to stimulation in muscle (i.e. decrease current required to induce a contraction with time). We wish to extend these studies by examining the time frame over which this differential sensitivity of nerve and muscle to stimulation change over time post-mortem and how we can increase the advantage of this “window” to the meat industry.

Aims:

1.Preliminary experiments will determine the stimulation parameters (low voltage and variable frequency and pulse width) that consistently evoke nerve-mediated and direct muscle stimulation

responses in sheep muscle in tissue bath set-up, using three preparations, one derived from predominantly fast twitch, one slow twitch and one from mixed skeletal muscle fibre bundles.
2.In-vitro experiments will be performed at set time points after induction of anoxic conditions (5min, 10min, 15min, 20 and 25min) using the above stimulation parameters in control and curare treated tissue preparations to determine the time frame during which the evoked contraction changes from nerve-mediated to direct stimulation.
3.This knowledge will then be transferred to whole carcass stimulation experiments where nerve vs. muscle stimulation settings will be used in Murdoch Universities experimental abattoir prior to application at the commercial operation in Katanning.

Objective 3: The effect of MVES on rate of pH decline and objective meat quality

The utilisation of electrical stimulation systems to stimulate anaerobic glycolysis and increase the rate of pH decline in muscles (Sams 1998; Polidori et al. 1999; Devine et al. 2004) will assist in meeting the pH temperature window guidelines established by the Australian Sheep Meat Eating Quality (SMEQ) Program to ensure optimal eating quality. For meat designated for the air freight and domestic market the meat should reach pH 6 when the carcass is between 18-25oC.

A preliminary validation study evaluated the effectiveness of the MVS post-dressing unit at different current and pulse width settings on pH-temperature decline at WAMMCO International Abattoir in Katanning Western Australia. With significant proportion of WAMMCO product shipped as air freight it is essential that the product reaches a pH of 6 between 18-25oC according to SMEQ guidelines. However, Pearce et al. (2006) demonstrated that only 42% hit of carcasses with the pH temperature window, with no unstimulated carcasses hitting the window. The lower percentage hitting the window can be attributed to the very fast chilling regimes at WAMMCO which will result in a lower temperature at which the carcass hits pH 6.

To reach the desired pH temperature window it appears necessary to increase the stimulation response to increase the pH drop and counteract the fast temperature decline. The study by Pearce et al (2006) did show that there was no effect of high currents and pulse widths on pH decline which may be attributed to the late application of stimulation when any stimulation response is dependant on direct muscle stimulation alone. However, other methods which may be more responsive at such a late stage of application post mortem were briefly touched on by Pearce et al. (2006) such as changing frequency and modulating the stimulation on/off times.

This study will investigate the alternative settings on the MVES to increase stimulation response and increase pH decline in the presence of the fast chilling regime at WAMMCO. The settings

which result in the greatest stimulation response will be evaluated for tenderness, colour and drip loss tests to ensure these settings result in optimal eating quality.

Aims:

1.To determine how different frequency and modulation settings on a medium voltage electrical stimulation unit affects stimulation response, subsequent pH decline and objective meat quality.

Methods:

1)This project will firstly test a variety of new setting combinations to increase the stimulation response represented by an increased rate of pH decline by alternating frequency and modulation settings.

2)The objective meat quality, ultimate pH, tenderness, and drip loss will then be assessed on the setting that results in the optimal stimulation response.

Objective 4: Determine the action of various neuromodulators at the neuromuscular junction under physiological conditions in response to different electrical stimulation parameters. This will allow us to better understand the nerve and muscle responses to the applied electrical current and will promote our basic understanding of neuromuscular junction signalling.

In skeletal muscle, acetylcholine is the sole chemical transmitter responsible for transmission across the neuromuscular junction. However, at the neuromuscular junction, various substances modulate the contractile responses by both pre- and postsynaptic mechanisms. These modulators include adenosine triphosphate (ATP), nitric oxide (NO) and calcitonin gene related peptide (CGRP). These modulating agents differ in the location of their origin and active sites, but all influence the events at the neuromuscular junction, with further possible sites of action downstream. The majority of research investigating skeletal neuromuscular transmission has been carried out in physiological environments. There is an increasing need, however, to study this event under varying conditions, as it will allow a better understanding of the neuromuscular junction and skeletal muscle contraction. We hypothesise that under hypoxic/ischaemic conditions the role of these neuromodulators is enhanced. This study will have application to both the meat industry and medical situations, where altered blood flow or reduced available oxygen can have profound effects on skeletal muscle activity. The experimental approach will involve the use of an isolated nerve muscle preparation developed in rats (adapted for sheep to ensure optimal application of knowledge to the meat industry).

Aims:

2.Analyse and compare the expression neuromodulators and receptors in sheep and rat, fast and slow twitch fibres.
3.Determine the action of various neuromodulators under physiological conditions and establish the mechanism by which they modulate synaptic transmission and the excitation-contraction coupling process.
Methods:

1) Immunohistochemical techniques will be used to assess the types of neuromodulators present in the neuromuscular junction in both slow and fast twitch skeletal muscle fibres.
2) The response of nerve-muscle preparations, as described above, will be tested under physiological conditions, using pharmacological agents to mimic or antagonise the neuronal release of CGRP, ATP and NO, and determining subsequent contractile responses to nerve-mediated and direct electrical stimulation of the skeletal muscle.

Objective 5. Define the responses of muscle and nervous tissues to complex high frequency electrical waveforms that can be utilised to immobilise animals for shackling but not prematurely causing pH decline.

Previous research by Daly et al. identified unexpected responses to high and variable frequency waveforms that involve the ability to manipulate the intensity of responses to electrical stimulation of muscles and nerves. The aim of these experiments is to evaluate the physiological basis of these phenomena.

Observationally, stimulation at high frequency produces a reduced force during the tetanus, which then decays rapidly. In the carcass this is seen as a tetanic contraction that is rapidly followed by a flaccid paralytic state and can be utilized as a period in which the animal can be safely shackled. Two other situations which have demonstrated unusual behaviours are:

1: Use of a frequency sweep soon after slaughter, from high to low frequency (range 10k Hz to 50 Hz) failed to produce an increase in muscle force as the frequency fell. This was done using current levels well over that required for maximal nerve-mediated responses and over a period of time much less than that required to produce significant fatigue.

2: A stepwise change in stimulation frequency from high to low frequency (2K, 500 and 50 Hz) in cattle before dressing using 3 seconds at each frequency produced responses at 2000 and 500 Hz as expected, but the response at 50 was either absent or much lower than expected.

These unusual behaviours may result from events at the neuromuscular junction or within the muscle due to direct muscle electrical stimulation. One theory explaining the effect of high frequency stimulation on carcasses is that stimulation of the nerves innervating skeletal muscle produces a temporal 'non-propagating' state due to their inability to follow such a high frequency pattern, possibly due to a mechanism affecting the refractory period or membrane potential, thus inhibiting the initiation of subsequent action potentials. However, other studies that examined changes occurring in functioning muscle due to events beyond the neuromuscular junction (in human muscle samples), have demonstrated that high frequency stimulation produces a loss of force in muscle accompanied by an increase in the excitation threshold of muscle. Various studies have shown that this may be due to a failure of transmission across the neuromuscular junction , although muscle samples directly stimulated (isolated and curarized muscle preparations) show a similar sensitivity to high frequency stimulation

We hypothesise that the high frequency stimulation used on carcasses interferes with:
a) the initiation of subsequent action potentials due to the nerves inability to follow such a high frequency pattern, possibly due to a mechanism affecting the refractory period of the action potential; or
b) the sarcolemmal excitability of muscle, thereby slowing the action potential waveform and increasing the excitation threshold of the muscle.

Aim:
Determine the mechanisms by which very high frequency stimulation (~2000-5000hz) and certain pulse widths produce this response in carcasses. A purpose built high frequency stimulator will need to be built for these experiments.

Methods
1) set-up nerve-muscle preparations under physiological conditions (temp, oxygen, pH) and determine twitch and tetanic responses to various high frequencies and pulse widths including increasing and decreasing ramp of stimulation frequency
2) determine the contribution of nerve-mediated vs. direct muscle stimulation by the use of curare (neurotransmitter blocking drug) under these high frequency stimulation conditions.
3) change the set-up to an anoxic/ischaemic nerve-muscle prep (with tissues bathed in paraffin oil) and repeat the above stimulations.

Results, Discussion & Conclusion

Refer to the supporting documents for detailed papers for each of the milestones (see Appendix):

Appendix A – (Milestone 1)

Appendix B – (Milestones 2)

Appendix C – (Milestones 3)

Appendix D – (Milestones 4)

Appendix E – The effect of stretch and temperature decline on the force of muscle contraction and the commercial implications for hot-boning meat production (Milestone 5&6)

Appendix A -

Refer to supporting documents A.MQT.0025 - A physiological understanding of new generation post slaughter electrical technologies (Milestone 1)

Appendix B -

Refer to supporting documents A.MQT.0025 - A physiological understanding of new generation post slaughter electrical technologies (Milestone 2)

Appendix C -

Refer to supporting documents A.MQT.0025 - A physiological understanding of new generation post slaughter electrical technologies (Milestone 3)

Appendix D -

Refer to supporting documents A.MQT.0025 - A physiological understanding of new generation post slaughter electrical technologies (Milestone 4)

Appendix E -

Refer to supporting documents A.MQT.0025 - The effect of stretch and temperature decline on the force of muscle contraction and the commercial implications for hot-boning meat production (Milestones 5 & 6)