final report

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Auditing guidelines for minimising cold shortening in sheep meat

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1 Executive summary

The aims of this project were to:

1. Evaluate electrical stimulation compliance at Sheep CRC participating plants (Part A).
2. Conduct electrical stimulation optimisation testing to improve the level of pH-temperature compliance (Part A).
3. Develop industry relevant recommendations on how to assess and rectify electrical stimulation compliance (Part B).

Background

As part of the Sheep CRC Information Nucleus Flock (INF) slaughters over 2 years in 2007 and 2008 it was identified that a number of key abattoirs in Australia which process lamb and sheep had medium voltage electrical stimulation (MVS) units that were operating sub-optimally. This meant they could not comply with the pH-temperature guidelines developed by the SMEQ program as adopted by MSA. These abattoirs had poor pH decline performance and thus a low proportion of carcasses reaching pH6 between 18-35°C.

A collaborative project directed by Sheep CRC and funded through MLA and AMPC was developed to identify reasons for the poor pH decline and optimse the stimulation units. It is important to note that many of the MVS units had been installed in abattoirs with little or no follow up or pH decline testing to determine the optimal settings for their plant and the machines have been left on factory settings. The project investigated possible causes for the sub-optimal performance and developed optimal stimulation settings for each abattoir. It has also analysed data to investigate the robustness of the current method for predicting the number of carcasses which “hit” the window and investigated areas for improvement in the audit process.

Part A: Detailed study of Sheep CRC participating abattoirs and development of testing and auditing protocols

Sheep CRC kills in 2007 and 2008 enabled an evaluation study of the current performance of medium voltage stimulation (MVS) units at five Australian plants. The rate of pH and temperature decline and the proportion of carcasses that achieved a pH of 6 between carcass temperatures of 18-35°C were used to assess electrical stimulation compliance. The results identified that at four CRC participating abattoirs which process a significant portion of Australian lamb and sheep meat the electrical stimulation units did not enable compliance with SMEQ pH-temperature guidelines as adopted by MSA. These abattoirs had poor pH decline performance and thus a low proportion of carcasses reaching pH6 between 18-35°C.

The average level of compliance at the four abattoirs was 16.6%. At the fifth abattoir the pH decline response was optimal with compliance at 76%. The positive result for this latter abattoir is a reflection of the significant amount of input and investment previously made so as to achieve an optimum setting.

At three of the four poorly performing Sheep CRC participating plants, a process to address the low level of compliance was undertaken that involved an initial assessment of the stimulation unit, testing of the unit and optimisation of the unit.

The process of optimising stimulation and rectifying any problems with the stimulation units resulted in an increase rate of pH-temperature compliance from an average of 16.6 to 81% at three of the abattoirs.

This study demonstrated that testing and auditing of MVS units is very important to ensure that optimal electrical stimulation response is achieved. At the 3 plants which underwent

stimulation optimisation, an individualised approach was taken to determine the reasons for poor compliance and settings tests were optimised on an individual basis.

These abattoirs now have electrical stimulation units programmed to an optimal setting and monitoring of product quality under the new settings will be ongoing. It is essential that abattoirs must take responsibility for their stimulation units and conduct regular in-house pH testing to ensure the stimulation unit is working optimally.

Part B: Preliminary Recommendations

The overall goal of project A.MQT.0044 was to provide industry relevant recommendations on how to assess electrical stimulation compliance and accurately determine the percentage of carcasses that meet the pH-temperature window of pH6 between 18-35°C. These recommendations could form the basis of a system which could be used for quality control at individual abattoirs to allow these abattoirs to detect non-compliance of their electrical stimulation technology.

It is important to note that this aspect of the project is ongoing because detailed statistical analysis has revealed that more extensive investigation needs to be undertaken and thus at this stage only preliminary recommendations can be made. The objective in the coming months is to confirm the recommendations listed below with a significant biometrical approach.

The analysis of data in the current project was based on the application of linear regression to pH/temperature data for each individual carcass as used in the Sheep CRC. Analysis to date has indicated that this method will create a bias in the estimate of how many carcasses 'hit' the window and that an alternative approach based on random regression may be preferred. At this stage the following preliminary recommendations are made, but these are subject to ongoing critique and further statistical development.

Recommendations:

1. Currently there is no target level of compliance for the pH-temperature window across industry. The only recommendation is that 90% of the carcasses must have an ultimate pH ≤ 6.00, yet this is not measured, but is assumed from decline data. A target compliance level needs to be determined and applied at an industry level.
2. The effective operation of the MVS units is indicated by installation of an electrical warning system for each electrode.
3. If **point 2** can not be achieved then as part of the audit process MVS units are switched off and the pH/temperature declined compared to when the unit is operating for a sample of carcasses from the same lot.
4. A random regression method is applied to pH/temperature data so as to determine the number of carcasses that have 'hit' the window.
5. For audit purposes 50 carcasses are sampled at anyone time and more than one consignment is represented.
6. A greater frequency than monthly measurement is applied for auditing purposes.
7. A training module is developed to encompass the use of the random regression approach, pH measurement, principles of electrical stimulation and other electrical technologies.
8. A system is developed to enable companies to access technical support for the optimisation of MVS units whether for use with sheep or beef.

Contents

2 Background

Electrical stimulation is now an important component of sheep meat processing in Australia (Hopkins *et al.* 2008). Electrical stimulation not only reduces the variability in sheep meat eating quality (Hopkins and Toohey 2006), but also enhances the proportion of meat reaching required pH temperature guidelines. Achieving pH-temperature compliance is a feature of the Meat Standards Australia (MSA) lamb and sheepmeat program which underpins the eating quality of lamb. The MSA program identified that for optimal eating quality the meat destined for the domestic or overseas (air freight) markets should reach pH 6 when the carcass temperature is between 18-25^{o}C (Thompson *et al.* 2005b) and the range was subsequently increased to 18-35^{o}C. Based on the results of the MSA program a large percentage of Australian processors have installed new generation medium voltage electrical stimulation system (MVS) units to obtain the necessary pH temperature decline to comply with the pH temperature specifications (Hopkins *et al.* 2008).

The MVS units are composed of a bar of segmented electrodes which ensure that only one carcass contacts the electrodes at any one time as they travel along the electrode bar at chain speed. The current remains constant and the voltage is varied (peak 300V) by controlled electronics which determine the resistance of the carcass and this feed back system alters the voltage accordingly as described by Hopkins *et al*. (2008). The mode of operation is very different to the traditional high voltage systems (HVS) which apply a fixed voltage averaged across all carcasses being stimulated (Devine *et al.* 2004). The results of Shaw *et al.* (2005) clearly showed that MVS did achieve comparable results to a HVS system with the production of lamb meat with a similar tenderness and eating quality level. However at a number of the abattoirs that had installed the MVS unit's no further follow up or routine monitoring of performance had occurred.

It was identified that lambs slaughtered at a number of Sheep CRC cooperating plants had a poor pH decline performance for 2007 and 2008 drop Information Nucleus bred lambs and thus a low proportion of carcasses reaching pH6 between 18-35°C. The results from the Australia wide assessment are shown in Table 1.

Sheep CRC plants identified for critical auditing- have not prior to this report had any pH testing done to determine the effectiveness of their stimulation units. Further to this one of these plants kills some lambs under the MSA brand which requires testing and verification of the rate of pH decline. The other abattoir identified for auditing was abattoir B. Studies on pH decline have been conducted at abattoir B in previous years (Toohey *et al.* 2006; Hopkins *et al.* 2008) and the plant found to achieve acceptable compliance. However this plant requires an upgrade to the stimulation unit to work optimally given an increase in chain speed and no further optimisation can be achieved until the unit is upgraded.

In Table 1 the excellent performance of the electrical stimulation unit at Abattoir E is highlighted. This site had the highest percentage in the pH-temperature window. The reason for this is that extensive pH testing has been conducted with this plant and an optimum setting for their production system has been identified.

The poor pH response at Sheep CRC participating plants was a driving force for the initiation of this project to develop key variables and testing protocols for the assessment of stimulation response through pH decline. The protocols were used during plant visits to (1) assess current electrical stimulation settings and requirements and (2) develop an appropriate stimulation dose to result in the maximum number of carcasses reaching the pH temperature window. It was expected that the stimulation dose would be individual for each plant.

The overall goal of this study was to provide industry wide recommendations on how to assess electrical stimulation compliance and accurately determine the percentage of carcasses that meet the pH-temperature criterion of pH6 between 18-35°C. These

recommendations are to form the basis of a system which could be used for quality control at individual abattoirs to allow these abattoirs to detect non-compliance of their electrical stimulation technology. As part of developing these industry recommendations, a statistically rigorous assessment was conducted to validate the method to calculate the percentage of carcasses that meet the pH-temperature guidelines.

Table 1. Sheep CRC Information Nucleus kills across sites- 2008 and 2009 (2007 and 2008 drop) results

Site	n	pH at 18°C	Temp at pH6	% In window (reaching pH 6 between 18-35°C)
Abattoir A	473	6.26	16.6	37.8
Abattoir B	629	6.05	16.2	48.2
Abattoir C	413	6.12	14.2	33.2
Abattoir C	296	6.14	13.9	31.2
Abattoir D	367	6.18	13.2	14.5
Abattoir D	389	6.31	11.1	3.0
Abattoir E	759	5.96	21.5	66.0

The aim is to achieve a pH18 of less than 6.00, between 18-35°C for pH6TEMP and a higher number as possible for the percentage in the window (pH6W).

2.1 Milestones

Milestone	Achievement Criteria	Due date
2	Undertake comparative studies across 4 plants to establish a set of auditing procedures which are consistent with all sites and be applicable to the wider industry. Studies implemented to demonstrate effectiveness of procedures to eliminate cold shortening.	1 June 10
3	Prepare report providing generic guidelines for improving the status of cold shortening in sheep processing plants.	30 June 10

3 Part A: Milestone 2: Detailed study Australian CRC participating abattoirs and development of testing and auditing protocols

This stage of the project aimed to develop (1) testing procedures to evaluate across a number of plants the effectiveness of electrical stimulation and (2) begin developing these testing procedures into industry applicable auditing recommendations for the future assessment of electrical stimulation performance.

The approach to identifying testing and auditing procedures involved:

1. Establishing methods to measure and assess pH decline and therefore stimulation response.

2. Problem solving at the individual plants to identify reasons for poor stimulation response. The stimulation unit was thoroughly checked and the currently used parameters recorded.

3. Understanding key logistical and processing variables at the plants- such as chain speed, chilling regimes, product classifications.

4. Conducting settings tests to evaluate the best setting for the individual plant.

5. Discussions with the plant during the testing phase to identify requirements for electrical stimulation, the development of pH testing and auditing protocols. The results of the testing were also discussed.

3.1 Measuring and assessing pH and temperature decline

3.1.1 Measuring pH decline

To determine electrical stimulation performance through pH decline the methods developed by the CRC for Sheep Industry Innovation (Pearce 2009; Pearce *et al.* 2010) for the evaluation of pH decline were applied. The aim was to determine if the electrical stimulation at the plant was sufficient to achieve a carcass pH of 6 over the carcass temperature range 18-35°C.

The pH and temperature of each carcass was measured 4 times post-slaughter: (1) as soon as possible after slaughter at around 35°C, (2) When the carcasses reached ~ 20°C (3) When the carcasses reached ~ 12°C and (4) Ultimate pH: 24h post mortem (PM). The assessment of ultimate pH is important because it indicates if the carcass is a 'dark cutter' i.e. the pH doesn't drop below pH6.

Muscle pH was measured using an Orion 250A pH meter (Cat. No. 0250A2, Orion Research Inc., Boston, Masset, USA) with either a glass body, spear-tipped probe (Cat. No. 8163BN, Orion Research Inc., Boston, Masset, USA) or a temperature compensating meter and probe (TPS Australia, Springwood, QLD, Australia). Muscle temperature was measured using a stainless steel cylindrical probe attached to the pH meter. The pH meter was calibrated before use and at regular intervals using buffers of pH 4 and pH 6.8 at room temperature. The pH and temperature measurements were taken in the left portion of the m. *longissimus thoracis et lumborum* (LL) muscle at the caudal end over the lumbar/sacral junction. About 19-24 h after slaughter, ultimate pH and temperature of the LL was determined. The pH meter was calibrated at chiller temperature (approximately 2°C).

3.1.2 Assessment of pH decline

One of the projects aims was to define the assessment of pH decline to determine the effectiveness of electrical stimulation.

The rate of decline in pH and temperature during the first 24 h PM was defined by this project as:

1. The pH of the carcass when the carcass reached 18^{o}C (pH18)
2. The temperature of the carcass when the carcass reaches pH6 (pH6TEMP)
3. From this information each carcass was then assessed to determine if the carcass reached a pH of 6 between a carcass temperature of 18-35^{o}C (pH6W) where 1 = yes and 2= no.
4. The first pH measurement (initial pH) gives an indication of the strength of the stimulation. Carcasses which reach a lower initial pH have received a larger dose of electricity.

For effective stimulation: the aim is to achieve a pH18 of less than 6.00, between 18-35^{o}C for pH6TEMP, a higher number as possible for pH6W and a ∆pH greater than 0.5

A linear regression procedure was used to derive the relationship between post-stimulation pH and temperature to allow the calculation of pH18 and pH6TEMP. This process was conducted individually for each carcass for each treatment within each consignment. The initial pH was taken as the very first pH reading taken at an approximate carcass temperature of 35°C. This linear regression algorithm was then used to calculate the pH at 18^{o}C and the pH6TEMP and subsequently the carcass was evaluated to determine if it reached a pH equal to 6 between 18-35^{o}C. The ultimate pH of the LL was specifically used to determine if the carcass had a high pH i.e. the ultimate pH of the LL was above 6. If so; no value for pH6W was recorded.

It was not possible to calculate pH6TEMP for all carcasses. Some carcasses with a slow pH decline reached their ultimate temperature before they reached pH6. These carcasses were given blank values for pH6TEMP. It was however possible to calculate a value for the pH of the carcass at 18^{o}C. The pH18 value was used to determine the window compliance pH6W.

These methods are different to those used by MSA who take pH readings until the carcass has reached pH6 and none thereafter (i.e. no ultimate pH readings). Often MSA graders would only take 3 readings, but on occasion up to 5. Taking the 3 pH readings is sufficient to allow calculation of the rate (slope) of pH decline and therefore further assessment of pH decline (see next section).

3.2 Evaluating the pH response using different stimulation settings at CRC participating plants

Of the 4 poorly performing CRC participating plants, 3 abattoirs (**CRC participating abattoirs- A, C and D- Table 1**) were identified as plants requiring intervention to improve their electrical stimulation compliance using the following steps.

1. Problem solving at the individual abattoir to identify reasons for poor stimulation response. The stimulation unit was thoroughly checked and the currently used parameters recorded.

2. Understanding key logistical and processing variables at the abattoirs - such as chain speed, chilling regimes, and product classifications. These are listed in Table 1.

3. Evaluation of the pH decline response over different levels of current and pulse width (referred herein as settings tests) to determine the best stimulation setting for the individual abattoir.

3.2.1 Problem solving and current unit set-up

Most of the electrical stimulation units were installed prior to Chris Mudford's employment at Realcold. When first visiting the plant with Chris Mudford from Real Cold- the first phase was to do a complete survey of the machine to confirm the setup on the unit and check for any operational problems. This included checking that all the module bars were working correctly

and were delivering the correct output as specified in the electrics of the system. The earthing, grounding and shorting potential of the unit was also assessed.

The next step was to establish the current settings on the machine. The current medium voltage units have a control dial which allows up to 6 different settings to be programmed on the units. When the stimulation systems were first installed- the 5 settings on the machine were custom factory settings. In most cases- no machines were using the same settings.

3.2.2 Plant processing requirements

To understand the plants requirements for electrical stimulation it is important to know the plants processing requirements and current practice. Understanding these factors can further help establish an optimal stimulation practice.

Important baseline information was determined at each plant;

- Chain speed: this established if the number of electrodes in the stimulation unit was sufficient for the chain speed- a 30-35sec time period on the stimulation unit is essential for optimal stimulation. Some plants had changed their chain speeds since installation and if the chain speed had increased then a higher number of electrodes were most likely required.

- Obtain chilling profiles for all chillers: to determine how hard the chilling regime was at plant. Those plants with a faster chiller regime require a higher stimulation dose to achieve the pH-temperature window compliance. Temperature loggers were used to record the temperature profiles of the chillers.

- Test for wind speed in chillers: relates to the chilling profile.

- Determine time from kill to boning: the shorter the time it is more likely the chilling regime is faster and therefore a greater stimulation response is required.

- Understand product being processed at plant - % lamb vs. mutton: both have different stimulation responses. Does the stimulation need to be specifically designed for each product?

- Determine product classifications and key markets: if they have a high percentage of export product with a long shipping time and minimal domestic product- the stimulation response to achieve the pH window of 18-35°C can be reduced to achieve the export market conditions as ageing will contribute to the final quality of the product and in this case the pH window under MSA changes to 8-18°C.

3.2.3 Discussions with the plant

Day one:

- Discussed the machine in general with the plant.

- Obtained feedback on their product performance and quality to date.

- Discussed what they are hoping to achieve with their units.

- Discussed the logistics and processing questions mentioned above such as chain speed, chilling regime, the product being processed and key markets.

Day two:

- Undertook testing and reported pH6TEMP, pH18 and the percentage in the window.

- Identified a setting that would result in a high percentage of carcasses in the window at the correct pH-temperature range. This setting was then discussed with the plant including what they could expect to achieve from using this setting.

These discussions with the plant were an essential part of identifying suitable electrical stimulation parameters and developing relationships to ensure an ongoing auditing program.

3.2.4 Settings tests

Stimulation settings tests were conducted over several consignments to incorporate a range of carcass types and the carcasses were also placed into the same chiller to minimise the effect of chilling on pH-temperature decline. At each plant the chiller that would result in the fastest temperature decline was used. This approach was used because the proportion of carcasses meeting the pH-temperature window of pH6 between 18-35ºC would be at the lowest in this chiller and it was assumed that a higher proportion would be observed in warmer chillers (Toohey *et al.* 2008).

It is important to note that all settings tests were conducted after problem-solving activities had been conducted and any necessary modifications to the stimulation units (see results) had been made.

The settings tested in general were as follows and are described in Table 2:

- No Stimulation: to establish a base line for the consignment.
- Initial setting: to demonstrate the impact of changing settings compared to the currently used settings.
- Highest pulse width and current: to record the maximum stimulation response on the unit
- A more moderate current and pulse width setting- to determine whether the high setting is causing over-stimulation and therefore heat shortening.
- Low current and pulse width setting: including a low dose allows for a range in stimulation response when compared with the high and moderate settings.
- Modulated setting: 2.5ms (ms = pulse width), 1A, 10, 15, 25, 10, 15, 25 Hz (Hz= Hertz) across 6 electrodes: Pearce *et al.* (2008) demonstrated that this setting could result in a maximal contractile potential and possibly cause fibre breakage of the muscle fibres without causing too rapid a decline in pH.

Specific details of the consignments tested and the electrical parameters used are given in Table 2. Both metal and plastic gambrels (hooks that hold the carcass on a moving slaughter chain) were compared.

At Abattoir A following the optimisation tests a Sheep CRC slaughter was conducted for lambs born as part of the 2008 INF drop. The pH and temperature decline were recorded as detailed in Part 1. The stimulation setting used was 2A, 2.5ms, 15Hz.

3.2.5 Data analysis

A REML procedure was used within Genstat (12th edition, VSN International Ltd Hemel Hempstead, UK) to analyse the response variables pH18 and pH6TEMP data within a plant. Setting (treatment) was included as a fixed effect and consignment as a random effect. A Tukey test was also conducted to examine treatment differences between treatments for pH18 and pH6TEMP.

Table 2. Processing and electrical stimulation specifications for the 3 abattoirs involved in Milestone 2.

	Abattoir A	Abattoir C	Abattoir D
Unit type	Pre-dressing MVS	Post-dressing MVS	Post-dressing MVS
Time stimulated PM	30 s	18 mins	27 mins
Chain speed when installed	6 carcasses/min	5 carcasses/min	7 carcasses/min
Current chain speed	6 carcasses/min	6 carcasses/min	11 carcasses/min
Stimulation time (sec)	25 s	35 s	25 s prior to install of 2 new module bars and 34s after install
Chilling regime and time to 7°C	Moderate-fast, 220 mins	Moderate, 310mins	Fast, 150 mins
Product type	Lambs for both domestic and export markets	Majority lambs for domestic market	70% lambs: 30% mutton. Majority for export
Time from slaughter to boning	18 hrs	24 hrs	15 hrs

Table 3. Electrical stimulation treatments and carcass numbers tested in milestone 2

	Abattoir A	Abattoir C	Abattoir D
Electrical stimulation problem identified	2 modules were inoperative	MVS unit only delivering half the current and current set too low	MVS unit current and pulse width set too low
Number of consignments tested	5	6	5
Number of lambs per consignment per treatment	10 (only metal gambrels)	10 (only metal gambrels)	7 x 2 = 14 (metal & plastic gambrels)
Number of lambs tested overall	5 x 10 x 5 treatments = 250	6 x 10 x 5 treatments = 300	5 x 14 x 5 treatments = 350
No stimulation- control	YES	YES	YES
Initial setting	Set on: Current 0.4A, Pulse width 1ms, Frequency 15Hz.	Set on: 0.2A, 1.5ms, 15Hz.	Set on: 0.4A, 1ms, 15Hz
Lowest setting	2A, 0.5ms, 15Hz	0.4A, 1.5ms, 15HZ	1A, 1ms, 15Hz
Moderate setting	2A, 2ms, 15Hz	0.8A, 1.5ms, 15Hz	NO
Highest setting	2A, 2.5ms, 15Hz	1A, 2.5ms, 15Hz	2A, 2.5ms, 15Hz
Modulated setting tested	YES	NO	YES
Average carcass weights (kg)	26.7	21.5	23.2
Average GR depth (mm)	19	9	15
Month tested	July	August	February

3.3 Results

Reasons for poor stimulation compliance were identified at abattoirs A, C and D (Table 3). At abattoir A, 2 of the electrodes were found to be inoperative and the modules were replaced. At abattoir C, the stimulation unit had undergone in-house modification and as a result was only delivering half the programmed current. This problem was rectified so as to deliver the specified current. At both abattoirs A and C the current and pulse width were also set too low to produce a viable stimulation response. The settings at Abattoir A were 0.4A, 1ms and 15Hz and settings at Abattoir C were 0.2A, 1ms and 15Hz. At both plants (Table 4) there was no significant difference between the no stimulation setting and the initial setting prior to optimisation. At Abattoir D the number of modules was insufficient for the chain speed. The unit was installed to cater for a speed of 7 carcasses/min, but the abattoir had increased the throughput to over 11 carcasses/min (Table 2). Two extra module bars were added to the unit which increased the stimulation period from 25 s to 34 s.

The setting tests (Table 4, Figure 1a) after the replacement of the new module bars at abattoir A identified that all stimulation settings resulted in an improvement in the percentage of carcasses reaching the pH-temperature window. There was a significant difference in pH18 and pH6TEMP between the stimulated and unstimulated treatments ($P < 0.05$). An optimal stimulation setting of 2A, 2.5ms and 15Hz was identified (Table 4). This setting is now is use at this abattoir. Subsequent measurement of 2008 born INF lambs demonstrated that the stimulation was working effectively with 80% compliance achieved (Table 4).

The setting tests at abattoir C (Table 4, Figure 1b) also demonstrated a significant difference in pH18 and pH6TEMP between the stimulated and unstimulated treatments ($P < 0.05$). An optimal setting was also identified at this plant. The setting of 0.8A, 1.5ms and 15Hz resulted in the best stimulation response achieving an optimal average pH at 18°C of under pH6 (Table 4). This setting was selected over the 1.0A setting, which gave a pH6TEMP of 33°C, on the basis that this may put the carcasses at an unnecessary risk of heat toughening (Thompson *et al.* 2005a). This lower current setting of 0.8A is now is use at abattoir C.

After the installation of 2 new module bars, an optimal stimulation setting was also identified at abattoir D (Table 4, Figure 1c) and a significant difference between stimulated and unstimulated treatments for pH18 and pH6TEMP was observed ($P < 0.05$). The setting of 2A, 15Hz and 2.5ms resulted in the best stimulation response achieving an optimal average pH at 18°C of under pH6 and this setting is now in use at abattoir D. This abattoir also had the fastest chilling regime of all the abattoirs examined. Plastic gambrels achieved a 30% better stimulation response compared to metal gambrels.

Table 4. pH-temperature decline testing at abattoir A, C and D - results for pH18, pH6TEMP, pH6W and initial pH.

	pH18*	pH6TEMP (°C)*	pH6W (%)*	Initial pH
		Abattoir A		
No Stimulation	6.37a#	11.9a#	20	6.81
2A, 0.5ms, 15Hz	6.12b	21.0b	57	6.33
2A, 2ms, 15Hz	5.97b	26.5c	48	6.28
2A, 2.5ms, 15Hz	5.70c	29.0c	**81**	6.16
Modulated 2A, 2.5ms, 10,15,25Hz	6.02b	22.2b	48	6.28
		Abattoir C		
No Stimulation	6.46a	7.5a	2	6.81
0.2A, 1.5ms, 15Hz	6.20a	6.5a	5	6.72
0.4A, 1.5ms, 15Hz	5.88b	26.6b	51	6.25
0.8A, 1.5ms, 15Hz	5.8b	29.2bc	66	6.19
1A, 2.5ms, 15Hz	5.77b	33.0c	**82**	6.08
		Abattoir D		
No Stimulation	6.23a	12.6a	0	6.64
Metal 0.4A, 1ms, 15Hz	6.34a	13.1a	22	6.53
Plastic 0.4A, 1ms, 15Hz	6.25a	12.7a	44	5.58
Metal 1A, 1ms, 15Hz	6.05b	18.5b	42	6.34
Plastic 1A, 1ms, 15Hz	5.99b	18.9b	64	6.4
Metal 2A, 2.5ms, 15Hz	5.94bc	21.1bc	**81**	6.26
Plastic 2A, 2.5ms, 15Hz	5.85c	22.4bc	86	6.17
Metal modulated 2A, 2.5ms, 10,15,25Hz	6.04b	20.8b	60	6.28
Plastic modulated 2A, 2.5ms, 10,15,25Hz	5.87c	24.6c	66	6.36
		Abattoir A CRC slaughter 2008INF		
Slaughter 1 (n=99)	5.76	28.0	84	5.99
Slaughter 2 (n=100)	5.74	29.7	72	6.1

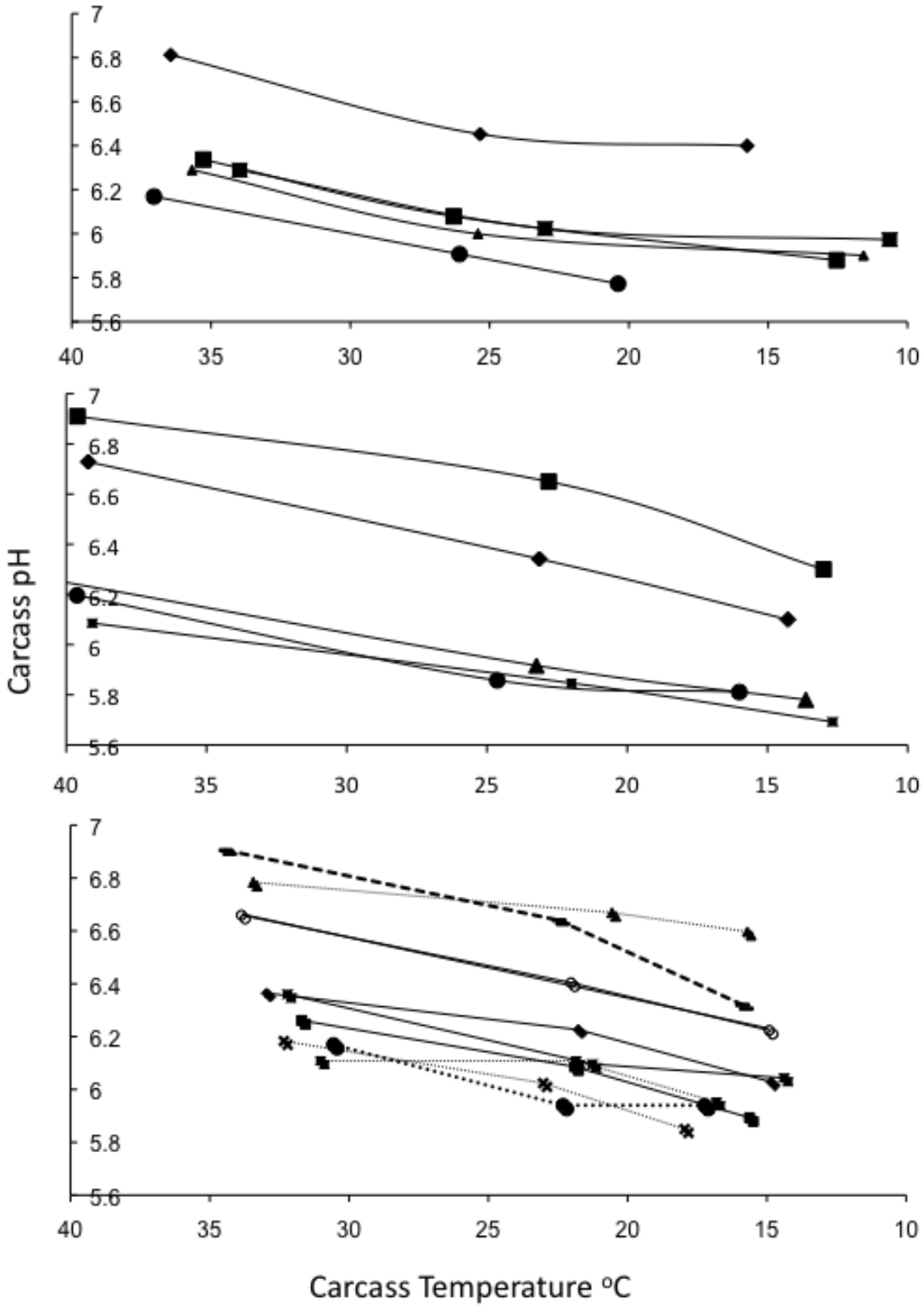
*The aim is to achieve a pH18 of less than 6.00, a pH6TEMP between 18-35°C, as high a number as possible for pH6W and an ΔpH which is 0.5 units different from the No Stimulation treatment

Values down the column within abattoir not followed by the same letters are significantly different ($P < 0.05$)

Figure 1a. pH and temperature decline of carcasses for Abattoir A in Part 2. υ No Stimulation, ν 2A, 0.5ms, 15Hz, σ 2A, 2ms, 15Hz, λ 2A, 2.5ms, 15Hz, ν Modulated 2A, 2.5ms, 10, 15, 25Hz.

Figure 1b. pH and temperature decline of carcasses for Abattoir C in Part 2. ν No stimulation, υ 0.2A, 1.5ms, 15Hz, σ 0.4A, 1.5ms, 15Hz, λ 0.8A, 1.5ms, 15Hz, ν 1A, 2.5ms, 15Hz.

Figure 1c. pH and temperature decline of carcasses for Abattoir D in Part 2. -▪- No stimulation, μ Metal 0.4A, 1ms, 15Hz, --σ-- Plastic 0.4A, 1ms, 15Hz, υ Metal 1A, 1ms, 15Hz, --ν-- Plastic 1A, 1ms, 15Hz, ν Metal 2A, 2.5ms, 15Hz, --λ-- Plastic 2A, 2.5ms, 15Hz, ν Metal Modulated 2A, 2.5ms, 10, 15, 25Hz, --6--Plastic Modulated 2A, 2.5ms, 10, 15, 25Hz.



3.4 Discussion

This study demonstrated that testing and auditing of stimulation units is very important to ensure that optimal pH decline results are achieved. At all 3 plants, reasons for their sub-optimal stimulation response were identified and these were primarily due to an increase in chain speed since installation, ineffective electrodes and a lack of subsequent testing of units after initial installation. Optimal stimulation settings were identified at all plants and all units set to these optimised settings.

The settings used on the post dressing unit at abattoir C were similar to those used in studies by Toohey *et al.* (2006) and at abattoir C a similar setting was used in the study to that used by (Pearce *et al.* 2006; Toohey *et al.* 2006; Jacob *et al.* 2008). Despite being identical units, the different settings used at the different plants reflect the placement of the units on the slaughter chain and the time between death and stimulation. The settings on the post dressing unit at abattoir C were able to illicit a similar pH response with a lower current compared to those used at abattoir D with stimulation at abattoir C occurring 9 mins earlier after death than abattoir D - 18 vs. 27 mins.

Electrical stimulation is a combined effect of direct stimulation of the nervous system, but also direct muscle stimulation. Direct stimulation of the nervous system will result in a greater force of contraction in the muscle compared to direct muscle stimulation (Asghar and Henrickson 1982). However the nerves have a reduced functionality for muscle contraction with an increase in time post slaughter (Bendall 1980; Chrystall *et al.* 1980; Asghar and Henrickson 1982). It is thus likely that the time difference between abattoir C and D is enough for a significant decline in stimulation via the nervous system. Chrystall *et al*. (1980) observed that at 30 mins post mortem there was very little effect of stimulation at high or low voltages on glycolysis when muscles were directly stimulated, indicating that the nerves had lost their capacity to trigger muscles to contract. This is due to either conduction failure or neuromuscular junction failure.

The settings used on the pre-dressing unit (abattoir A) were higher in current and pulse width compared to the study by Toohey *et al*. (2008). In the study by Toohey *et al*. (2008) the setting of 0.8A, 0.5ms and 15Hz was sufficient to illicit a similar pH effect (pH6TEMP of 24.8°C) as that observed in this study at between 2A, 0.5ms and 2A, 2ms (20.9 and 26.5°C pH6TEMP respectively). A likely reason for this discrepancy could be the different installation and operation factors of the pre-dressing stimulation units at the plants and issues such as electrical insulation and leakage which is difficult to measure. In this experiment at abattoir A the average carcass weight was 26.7kg compared to 21 kg in the study by Toohey *et al*. (2008). Lambs with higher carcass weights require a greater stimulation dose to generate the same stimulation response as lighter carcasses. The difference in stimulation responses and settings for both the pre and post dressing units detailed in this report and in previously published papers at different sites highlights the need to individually determine optimal electrical stimulation settings for an abattoir.

At Abattoir D, plastic gambrels achieved a 30% better stimulation response compared to metal gambrels and so the abattoir management plans to convert to plastic gambrels. It is possible that some voltage leakage was escaping up into the chain with the metal gambrels. However the plastic gambrels may result in an inadequate circulation of the current through the carcass driven by the force of the current being drawn into the rails about the gambrels (Chalcroft and Chrystall 1975). This has not been thoroughly researched and may be site specific.

It is important to note that abattoir D are planning to further increase their chilling rates. To improve stimulation response and to counteract the effect of the fast chill they are planning to install a pre-dressing stimulation unit which also has the benefit of potentially increasing blood collection at the start of the slaughter process (Hopkins *et al.* 2006).

There was a significant difference in the pH declines of the carcasses that were not stimulated between the different plants. This reflects the differences in temperature decline between the consignments and also the carcass types and quality (Pearce *et al.* 2006). This result indicates that it is essential to include a control/no stimulation treatment as part of all stimulation compliance testing within a consignment to understand the baseline level of glycolysis in the consignment and reflect carcass type and quality.

This study has also highlighted that it is essential that abattoirs take responsibility for their stimulation units and conduct regular in-house pH testing to ensure the stimulation unit is working optimally. Quality assurance staff may need more guidance to develop their testing procedures and understand the results. Monitoring of product quality under the new settings will be ongoing as part of further Sheep CRC slaughters.

3.5 Conclusions

This study demonstrated that testing and auditing of MVS units is very important to ensure that optimal electrical stimulation response is achieved. At the 3 plants tested in this study with poor electrical stimulation compliance, an individualised approach was taken to determine the reasons for poor compliance and settings tests were optimised on an individual basis. These abattoirs now have electrical stimulation units programmed to an optimal setting and monitoring of product quality under the new settings will be ongoing. The protocols developed from this study are being used to begin developing industry applicable and statistically valid recommendations for the accurate prediction of the proportion meeting pH-temperature specifications and therefore electrical stimulation compliance. This study has also highlighted that abattoirs must take responsibility for their stimulation units and conduct regular in-house pH testing to ensure the stimulation unit is working optimally.

4 Part B: Milestone 3: Preliminary Recommendations

The overall goal of project A.MQT.0044 was to provide industry relevant recommendations on how to assess electrical stimulation compliance and accurately determine the percentage of carcasses that meet the pH-temperature window of pH6 between 18-35°C. These recommendations could form the basis of a system which could be used for quality control at individual abattoirs to allow these abattoirs to detect non-compliance of their electrical stimulation technology whether they be MSA accredited or not.

It is important to note that this aspect of the project is ongoing because detailed statistical analysis has revealed that more extensive investigation needs to be undertaken and thus at this stage only preliminary recommendations can be made. The objective in the coming months is to confirm the recommendations listed below with a significant biometrical approach.

On the 15/6/10 the team had a phone hook-up with MSA and also met with Janine Lau from MSA on 25/6/10 to discuss current progress and get feedback on the analysis to date. The feedback has been positive, but the new approach to analysis requires verification and critique from fellow researchers. It should also be noted that discussion with MSA has revealed that:

1. For lambs consigned direct under MSA 20 head per category per month must be measured to determine compliance with the pH-temperature window. However **no** level of compliance is specified by the guidelines. Further to this if the lot is more than 20 carcasses, the first 20 that hit the chiller are measured and this is not the best approach to deriving the "true" picture of the pH decline of the lot. A random selection across the entire lot should be used.
2. For both saleyard and carryover lambs they must comply with the window, but again no compliance level is specified. Additionally 90% of the carcasses must have an ultimate pH ≤ 6.00, yet this is not measured, but is assumed from decline data.
3. The pH and temperature data for each lamb is recorded at set time periods and then averaged across all 20 carcasses to derive one line for pH-temperature and this is visually assessed to determine compliance.

The analysis of data in the current project has involved the application of linear regression to pH/temperature data for each individual carcass which is different to that used by MSA as specified above. Clearly MSA are keen to improve the approach to monitoring compliance and the team will work with MSA to deliver on the recommendations outlined below.

At this stage the following preliminary recommendations are made but these are subject to ongoing critique and further statistical development:

1. The effective operation of the MVS units is established by installation of an electrical warning system for each electrode.
2. If **point 1** can not be achieved then as part of the audit process MVS units are switched off and the pH/temperature declined compared to when the unit is operating for a sample of carcasses from the same lot.
3. Three pH/temperature readings be taken - Reading 1: 20 mins post-mortem (PM), Reading 2: 70 mins PM, Reading 3: around 240 mins PM. These equate to carcass temperatures of approximately 38, 29 and 15°C.
4. A random regression method is applied to pH/temperature data so as to determine the number of carcasses that have 'hit' the window.
5. For audit purposes 50 carcasses are sampled at anyone time and more than one consignment is represented.
6. A greater frequency than monthly measurement is applied for auditing purposes.

7. A training module is developed to encompass the use of the random regression approach, pH measurement, principles of electrical stimulation and other electrical technologies.
8. A system is developed to enable companies to access technical support for the optimisation of MVS units whether for use with sheep or beef.

To underpin these industry recommendations, the pH and temperature data generated from Milestone 2 was statistically analysed to develop methods and protocols to accurately determine the percentage of carcasses that met pH-temperature guidelines and therefore electrical stimulation compliance.

1. Check electrical stimulation unit is functioning correctly:

A common reason for poor MVS compliance was malfunctioning electrodes. Currently units have a red light (or similar) to indicate the unit is on, but this does not identify if particular electrodes are inoperative. At a recent visit to a plant 2 electrodes were found to be inoperative when checked by the plant electrician even though the system was deemed to be operating. **Additionally there does appear to a particular problem with shortage in pre-dressing sheep MVS units that is causing electrodes to malfunction which requires rectification.**

To improve monitoring each electrode could be wired with an individual light to indicate that it is operating correctly. This would then be part of the daily audit system.

If a lighting check system or similar is not possible/present then initial compliance testing should include a baseline (control) test by turning off the stimulation unit for a small number of carcasses within a consignment and conducting a pH decline. If there is no/minimal difference in pH decline between the non-stimulated and stimulated carcasses it is likely that the machine is not operative and servicing is required. Including a control/no stimulation treatment as part of all stimulation compliance testing within a consignment will also allow an understanding the baseline level of pH decline (glycolysis) in the consignment and reflect carcass type and quality.

2. Three pH-temperature decline readings will accurately determine the proportion hitting the window:

Preliminary analysis indicates that three pH-temperature decline readings may to be sufficient to get an accurate reflection of the proportion meeting the pH-temperature guidelines provided the random regression method (see below) is used to analyse the data.

The best precision was achieved when the ultimate pH was the third pH reading. However, this is not always an option for in house testing as often carcasses are boned before 24 post-mortem (PM) when a reliable ultimate pH could be expected. However, if the ultimate pH is not available high accuracy was achieved as long as the first reading was as soon as possible after slaughter - specifically when the carcass temperature was above 30°C and the following 2 readings are close in time. The best accuracy on the datasets tested equated to carcass temperatures of 38, 29 and 15°C.

Discussion on the use of 3 pH readings at specified temperatures with MSA has revealed it may not be practical to do it this way. Currently the MSA approach is to have plant operators take a reading at hourly intervals until the pH is below 6. The specific requirement to take measures hourly restricts the potential to apply our recommendation, but it also may be that measurement of carcasses 5 times during chilling (as specified currently by MSA) will be as effective provided the range in temperature and pH is wide. This will be investigated in future analysis using data provided by MSA.

3. Use a random regression model to calculate the proportion that meet the pH-temperature guidelines:

The current approach detailed in Parts 1 and 2 to determine the proportion of carcasses that meet the pH-temperature guideline is to fit a linear regression of pH on temperature for each carcass. The estimate of the proportion meeting the pH-temperature guideline is simply the mean number of carcasses that meet the guideline. If observations are taken on *n* carcasses, of which *x* meet the pH-temperature guideline, then an estimate of the proportion is given as *x/n*. This method will be referred to, as ***Estimate 1*** and this does not use information from other carcasses in the sample to improve the reliability of the estimate.

An alternative approach is to jointly fit regression lines to the carcasses assessed during compliance testing using a random regression model. Briefly, the model assumes that carcasses have on average a smooth trend in pH with temperature and that individual carcasses deviate, independently, about this average trend. These deviations for a carcass are modelled as linear in temperature, with regression parameters sampled from a bi-variate normal distribution with zero mean. Generally the smooth average trend is taken as linear though this is not necessary. Nor is it necessary to assume that carcass deviations are linear, though this is simpler and generally adequate.

Having fitted a random regression model to the carcasses within a consignment it is possible, based on the parameter estimates of the model, to assign a probability to each carcass that it individually met the pH-temperature criterion of pH6 between 18-35°C. The proportion of carcasses in the consignment that met the criterion is then estimated as the average of these probabilities. This estimate is referred to as ***Estimate 2***.

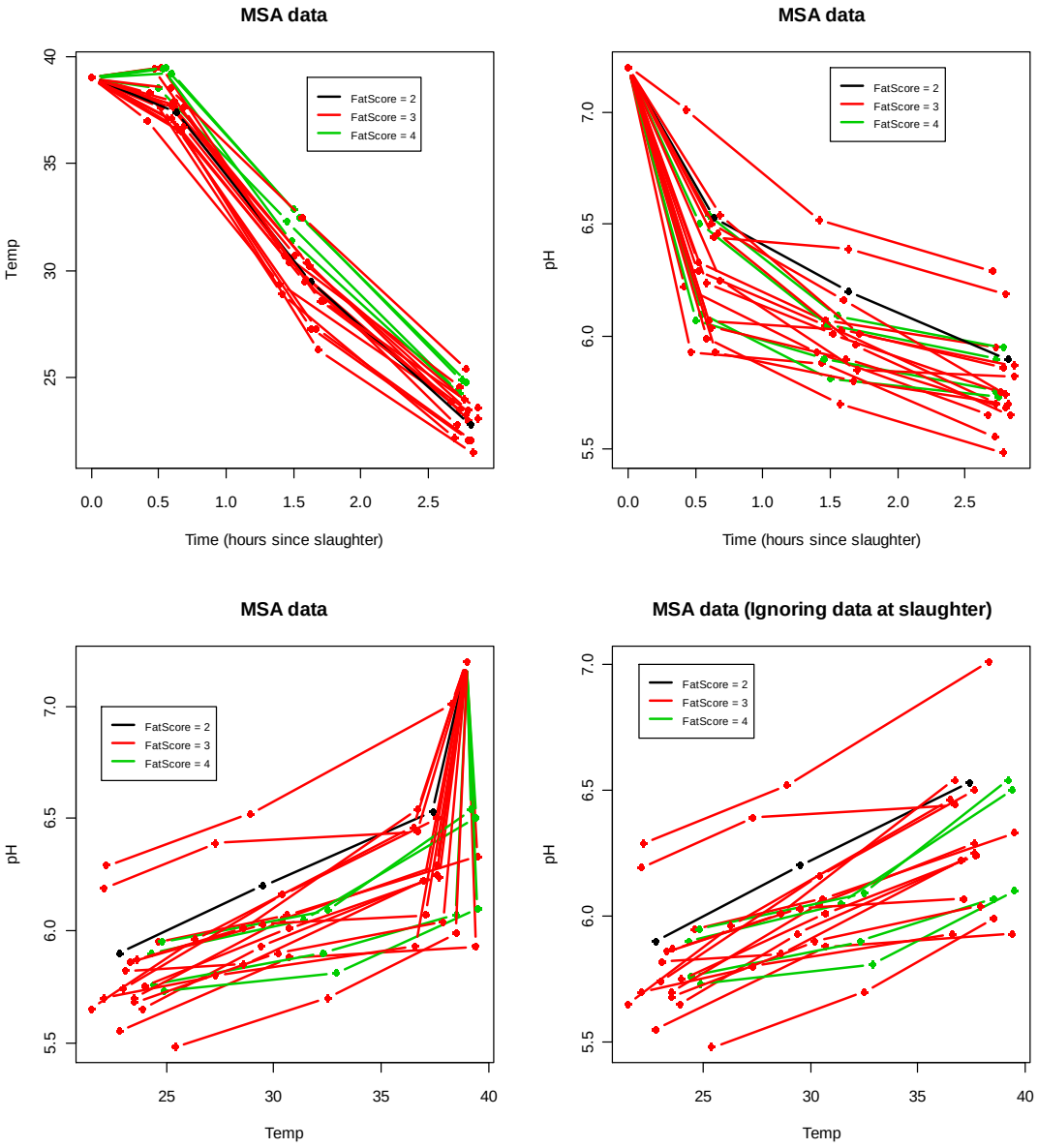
Estimates 1 and 2 were subsequently compared using data from the 2008 born INF lambs slaughtered at Abattoir B (Hopkins *et al.* 2009). This data comprised fifty-five lamb carcasses that were electrically stimulated with the same dose as the 2007 drop lambs as described in Table 1. Each carcass was measured for pH and temperature six times over a 24-hour period. In addition to pH and temperature the time of measurements was also recorded. This enabled good estimates to be obtained for the distribution of the decline in both pH and temperature over time across carcasses in this consignment. The estimate of the proportion of carcasses meeting the pH-temperature criterion for this consignment was 95%. Subsequently one hundred simulated data sets of three pH and temperature recordings on each of 55 carcasses were generated at times 20 mins, 70 mins and 185 mins PM from a distribution having parameters as estimated for the 2008 born INF lambs slaughtered at Abattoir B. Estimates 1 and 2, based on regression of pH on temperature, were then generated for each of the simulated data sets.

For the data set of Hopkins *et al.* (2009) a proportion of 0.95 (95%) of the carcasses are estimated to meet the pH-temperature guidelines of pH6 between 18-35°C. Both estimates 1 and 2 are in statistical agreement with this estimate when based on the regression of pH on temperature over the six measurements. However, when simulating data for the three times considered from a distribution with these parameters (in which case the true proportion is 0.95) the results are very different, Estimate 1 gave on average an estimate of 0.76 (s.e. 0.06) whilst estimate 2 gave on average an estimate of 0.95 (s.e. 0.04). Estimate 1 clearly has a negative bias in this situation whereas estimate 2 gives an unbiased estimate. Similar results have been found in other simulated data. This is in many ways not unexpected as estimate 1 will generally perform poorly when the range of results (pH and/or temperature) is small and only a limited number of reading are taken.

This aspect of the study has identified that improvements in the determination of the proportion of carcasses meeting specified pH-temperature guidelines can be made by the application of more rigorous regression methods.

This method can be demonstrated using actual MSA data on 20 lamb carcasses. This data can be viewed in the four sub-plots in Figures 2 and Figure 3:

- Temp versus Time (since slaughter, in hours) for each carcass
- pH versus Time for each carcass
- pH versus Temp for each carcass from slaughter till third measurement
- pH versus Temp for each carcass omitting data at slaughter



Figures 2. MSA pH vs. time and temperature

Below are plots of the data from Figure 2, but the estimate of the probability of each carcass passing through the window is given at the right end of the line joining its points calculated using the random regression model.

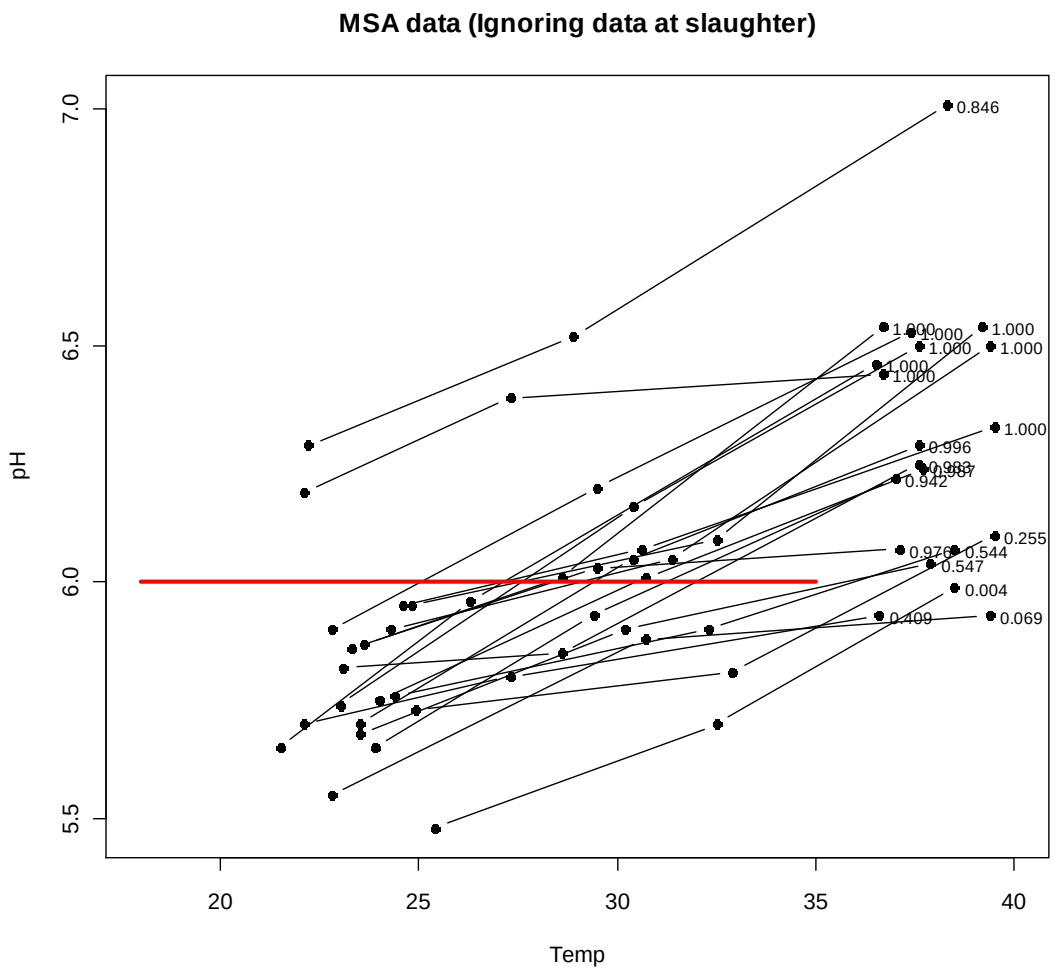


Figure 3. MSA data for pH decline with associated probability of "hitting' the window.

Fitting the random regression model to the data, ***ignoring the results at slaughter***, gives an estimate of the proportion of carcasses in this consignment that "hit the window" as **0.78 or 78%.** An estimate of the standard error associated with this estimate is 0.09, with a 95% confidence interval that the data falls between 60-96%. It should be noted the current MSA system assumes the starting point is common for each carcass, which is not based on measured carcasses and for this reason this data has been excluded from the analysis.

By contrast if regression based on a line of best fit for each carcass individually (as used by the Sheep CRC) then the proportion "hitting" the window is estimated as 60%. Thus it should be noted that the recommendation to switch to the random regression method will apply to Sheep CRC data also. It has been demonstrated that the current method produces a bias in the estimate that can be overcome by the application of random regression. Further examination of other data sets (MSA and Sheep CRC) will be undertaken to verify that this approach is sound and also compare this with the averaging approach currently used by MSA. This will include examining whether analysis of temperature/time and pH/time data is more appropriate for deriving the pH18, pHTEMP6 and the proportion in the window.

This random regression approach could be developed into a computer program which can easily be used by abattoir staff, MSA graders through to meat researchers. This computer program could be an update of the current MSA pH decline program or similar to the Sheep CRC pH decline calculation model. However this task will need significant input from Dr Remy Van de Ven (I&I NSW) a statistician with extensive understanding of pH decline modelling. This activity will need to be funded.

4. Conduct pH testing on 50 not 20 carcasses.

This project can demonstrate that increasing the carcass sampling number from 20 to 50 carcasses will result in greater prediction accuracy, but sampling greater than 50 would not result in a significant improvement at estimating the proportion “hitting the window”. Assuming the value for effective stimulation is 80% of carcasses “hitting the window” it could be expected that if the number of carcasses tested increases from 25 to 50 then there is a 25% reduction in the range of the confidence interval significantly improving the confidence that the proportion is 80%. We believe that this area requires further work and our current recommendation requires validation. Also by examining pervious data a recommendation on what the level of compliance should be can be made.

5. How often to sample over the period of a year?

Sheep CRC testing demonstrated a significant effect of kill date on pH response. This can attributed to both differences in product being killed and temperature decline. The differing responses to stimulation over kill days will influence the auditing frequency for abattoirs. Testing once a month may not give a true indication of how the system is working. Audits should be conducted to allow for variation in factors such as lamb source, weather, product type etc rather than be set to a fixed date only. To enable a cross-section of animals processed at the plant to be sampled it would be important to conduct pH testing over a number of different consignments. Further analysis to answer this question is required and our current recommendation requires validation. The required work under points 3-5 could be combined into one activity.

6. Training of abattoir staff

Overall we believe it is essential that abattoirs take responsibility for their stimulation units and schedule regular in-house pH testing to ensure the stimulation unit is working optimally.

On the basis that the random regression method is adopted then staff will need to be trained in how to apply this method. Obviously if a training package is developed then for MSA accredited abattoirs MSA can provide that training once they are trained by the appropriate personnel. Thus for plants that are MSA accredited regular electrical stimulation compliance testing could conducted under the MSA banner.

However, there are a number of plants that have MVS units installed that are not MSA accredited. Discussion with MINTRAC has confirmed that they can facilitate the training by using experienced personnel to deliver the training. This training it is proposed would also cover the measurement of pH, principles of stimulation and other electrical technologies. In this regard it should be noted that a small number of sheep abattoirs have immobilisation and electronic bleeding installed and so the application and benefits of these technologies also needs to be covered in the training. MINTRAC approached the Sheep CRC to review their electrical stimulation training manuals, so there is synergy in combining these activities. It should be noted that the Meat Technology Update (6/07 December 2007) produced previously for FSA by the Sheep CRC would also requirement revision. This would require funding. There are 2 personnel who have extensive experience in these electrical technologies who can satisfy this requirement Dr Kelly Pearce (Murdoch University) and Mrs Edwina Toohey (I&I NSW) with oversight from Dr David Hopkins (I&I NSW).

7. Optimisation studies

In the initial roll out of these new technologies both Murdoch University and I&I NSW were involved in a number of studies to help abattoirs optimize the performance of the units. This has been also undertaken in the current project as part of the problem solving approach. This has in the past involved testing for the effect on tenderness and meat colour. Clearly these are activities that

companies will not undertake themselves. The approach that should be adopted here requires more discussion, but one possibility is that companies can on a fee structure engage the services of the R&D organizations listed in point 6.

Maintenance and installation of electrical stimulation units in Australia

We recommend that the maintenance, installation and servicing be conducted by an Australian company. All the research conducted in the CRC/MLA/AMPC project would not have been possible without the assistance Chris Mudford from Argus RealCold. Chris is a highly skilled operator with extensive experience with a variety of different electrical stimulation technologies. With the imminent departure of Argus RealCold from the installation and servicing of these electrical technologies a strategy must be developed to address this issue. This technology has had remarkable uptake and put Australia at the forefront in methods to improve eating quality. We must not relinquish this position. It is our understanding that a number of other countries have also invested in these technologies.

Database and computer programs

The database which Argus RealCold holds on what is installed at each abattoir (both sheep and beef) should be secured by an appropriate body or another company that might take on this part of Argus RealCold business. This would also apply to the computer programs that are used to control the settings of each unit.

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6 Publications arising from this project:

Two papers on the results of this work have been submitted for the ICoMST in Korea and a full journal paper is being prepared to coincide with a Sheep CRC conference in October.

Pearce, K.L., Hopkins, D.L., Williams, A., Hocking Edwards, J. Jacob, R.J., Withers, R. Refshauge, G., Geesink, G., Warner, R.W. and Pethick, D.W. (2010). Assessment of electrical stimulation compliance at abattoirs using medium voltage electrical stimulation. *Proc. 56th International Congress of Meat Science and Technology.* Jeju, South Korea (in press).

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