Investigation of the rigor process of beef muscles and development of recommended aging times for key muscles

Siou Yong Kwan

A thesis submitted to Auckland University of Technology in fulfilment of the requirements for the degree of Master of Philosophy (MPhil)

2015

School of Applied Science

Table of Contents

List of Figures

List of Tables

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award or any other degree or diploma of a university or other institution of higher learning.

Signed:  .....

Date: .....06/07/15.....

Acknowledgements

I would like to express my outmost gratitude to the following institutions and people who have offered their tremendous support, guidance and cooperation.

Firstly, I would like to thank Silver Fern Farms Limited, especially Grant Pearson, the Technical Innovations Manager; and Philip Shuker, the Otago/Southland Regional Manager. They were the very first individuals who agreed on and supported my decision to studying part time while working full time. I would like to especially thank Grant Pearson, who mentored me patiently throughout the project. Not to forget my academic supervisor, Dr Owen Young, who understood the difficulty of commercial research and had given his outmost support and guidance to my study. Also, I would like to thank the joint-venture company Farm IQ Limited for sponsoring the project.

I would like to extend my gratitude to my work colleagues: Stephanie Smithson, Athol Hines and Malcolm Buchanan, who never got tired of me firing questions at them. Their support, experience and advice were invaluable. Furthermore, I would like to thank Clyde Daly and Nicola Simmons from Carne Technology, who have shared their industrial experience and knowledge with me. Also, I would like to thank Dr Martin Bader for his invaluable assistance in statistical analysis.

I would like to thank all individuals in the Finegand processing plant, who lent a hand and cooperated to ensure the success of this project. Also, I would like to thank the team at Finegand and the Belfast Central Lab for loaning the equipment (and even the lab) to me.

Lastly, I would like to thank my family and friends who supported me on my writing and encouraged me to strive for success.

Abstract

This thesis is aimed to evaluate three electrical stimulation systems in beef processing. A new setting has replaced the traditional 15 Hz immobilisation setting in all processing plants. However, the impact of the new setting on various meat quality parameters – pH decline rate, tenderness, drip loss, colour, etc. – was of interest. That is the first objective of this study. The second objective of this study is to understand the behaviour of representative meat cuts in response to the three stimulation treatments. The output from this work will be a comprehensive muscle profile database of commercial value.

36 pasture-fed steers (male castrates), aged 18 to 28 months and weighing between 280 and 330 kg were singly selected for the study. The subcutaneous fat thickness was between 2 and 4 mm. The steers were electrically stunned across the head only, bled and dressed according to Ministry for Primary Industries (MPI) requirements. The time at this point of the experiment was designated Day 0. Three stimulation treatments were devised: the traditional 15 Hz treatment is Stimul 1 and the new setting, 15 Hz and 400 Hz. is Stimul 2. Stimul 3 is Stimul 2 paired with Smart Stimulation. The 36 animals were randomly assigned to one of them.

Six pH and temperature measurements were taken from each carcass with a TESTO® 205 pH meter (Testo AG, Lenzkirk, Germany) at approximately hourly intervals from the start of chilling to about 8 hours post slaughter, then finally at 24 hours: the pHu. The pH meter was calibrated before use and at regular intervals using pH 4 and pH 7 buffers at room temperature (Thermofisher, New Zealand). The glass probe was inserted through the *longissimus* between the 12th and 13th ribs and into the core of the deeper *M. semimembranosus* on the hindquarter.

One day after slaughter (Day 1) six single muscles: *rectus femoris, longissimus dorsi, psoas major, gluteus medius, gluteobiceps* and *infraspinatus* were excised from one side of the carcasses. Depending on the size and orientation of each muscle, 2 or 4 submuscles (100 mm x 50 mm x 50 mm) were excised, trimmed of visible fat, and labelled as Position 1, 2 etc. from a defined site depending on the muscle. In summary, a grand total of 648 samples were collected in this study. Each muscle subsample was vacuumed packed and randomly assigned to one of six different aging

days, 1, 3, 5, 7, 14 or 21 days. At the prescribed day, the muscles were measured for drip loss, colour and tenderness.

Although the pH-temperature data were unfortunately limited for Stimul 1, historical data strongly suggested that there would be a severe risk of heat shortening, particularly in the deeper *semimembranous* muscle. By contrast, the pH-temperature results in Stimul 2 and 3 showed little tendencies for heat shortening. The 15 Hz and 400 Hz combination in Stimul 2 reduces the incidence of burst tenderloins and ecchymosis. Stimul 3 did demonstrate an ability to control pH decline rate in the first few hours but only in *longissimus dorsi* and not extended to *semimembranosus*. In the two indicator muscles, Stimul 1 had the most variable pH values, particularly at early times after slaughter. Variability was reduced in Stimul 2 and – especially - Stimul 3 (Smart Stimulation). Thus, Stimul 3 appears more attractive.

The stimulation treatments had no effect on drip loss, saturation, Hue angle and shear force, but the data reveal the rank order of drip loss amongst muscles is the same as saturation and Hue angle: *Gluteus medius* (RUMH, highest drip loss and most coloured) > *Gluteobiceps* (RUMC) > *Psoas major* (TEND) > *Rectus femoris* (KNUCK) > *Longissimus dorsi* (STRL)> *Infraspinatus* (BLD, lowest percent drip loss). The initial shear force values for *infraspinatus* (BLD), *rectus femoris* (KNUCK) *gluteobiceps* (RUMC), *gluteus medius* (RUMH) and *psoas major* (TEND) were below 10 kgF, and would provide an acceptable eating experience even one day after slaughter. The *longissimus dorsi* (STRL) showed a high variation of tenderness (between 5 kgF to 12 kgF). The tenderness variability of all muscles decreases with time and they would provide a good eating experience after 7 to 10 days after slaughter. The remarkably precise inverse relationship between the Japan colour score and L* showed that the colour tiles were as good, certainly cheaper and more reliable by virtue of their simplicity. This colour grading technique is currently in commercial practice by Silver Fern Farms.

Chapter 1
Introduction

The challenge for red meat industry

In the year ended June 2013, the top New Zealand export commodities were dairy, meat and wood, which accounted for 44% of the total export (Statistics New Zealand, 2013). Among the export goods, the red meat and co-products exports worth $6.3 billion, nearly $300 million more than in 2012. Therefore, red meat remains as one of New Zealand's major export earners (MIA, 2013).

Despite being a major export earner, the red meat industry faces many challenges. The main challenge is the time lag between production and processing, because the conversion of pasture into edible muscle (i.e. meat) takes months. The period between a farmer's decision to produce and deliver livestock to a meat processor can be over a year for lamb and at least two years for beef. Additionally, there are several months between livestock processing and consumption by overseas customers (MIA, 2013).

Due to the seasonal nature of the New Zealand climate, the supply of livestock is neither even nor continuous throughout the year. The supply trend is like a roller coaster ride: oversupply in summer and autumn, but an undersupply in winter. This seasonal mismatch of supply and demand creates pricing volatility. Unlike other manufacturers, it is impossible for the red meat processor to resolve this issue by simply holding back livestock and product inventory and then later adjusting their production based on market price fluctuations.

These challenges were evident following two seasons of unsatisfactory prices for farmers and financial results for meat processors in 2012 and 2013 (Silver Fern Farms, 2012, 2013). The current conditions have been aggravated by the ongoing conversion of land from sheep and beef farming to dairy farming; and a corresponding reduction in sheep and beef livestock numbers. These conditions have led to a call for structural reform of the red meat sector.

The call to restructure the red meat sector

In 2010, Silver Fern Farms Limited, PGG Wrightson, Landcorp Farming and the Ministry of Primary Industries established a jointly-funded seven-year programme to turn the meat industry's production-led approach into a market-led approach. The programme focused on responding to consumer needs through a pasture-to-plate integrated value chain. It is run as a joint venture through a purpose-created company called the Farm IQ Systems Limited. By the end of this Primary Growth Partnership (PGP) programme, it should grow New Zealand's Gross Domestic Product (GDP) by $1.1b in year 2017, accumulating to $8.8b by 2025. According to Farm IQ (2010), this value increase is crucial for the survival of the farmers and meat processors.

Under the PGP programme, Silver Fern Farms has identified a number of areas that could be evaluated: meat quality measurement, meat quality improvement, process optimisation, product traceability and meat yield measurement projects. This electrical stimulation thesis project is funded under the meat quality improvement and measurement categories.

Silver Fern Farms process evaluation

In 2011, Silver Fern Farms approached Carne Technologies to review their electrical processing protocols in all beef plants. The aim was to create a benchmark understanding of the current processing standards and to identify any areas for improvement. In Halal beef plants, with head-only stun, the animals are rendered unconscious and completely unresponsive to pain when the slaughter cut is made. By the time that the animals regain conscious from stun, the brain has ceased function due to oxygen deprivation through excessive blood loss. However, there are a number of reflexive movements mediated by the spinal cord after stunning. Violent kicking and walking movements will occur during the first few minutes after stunning, especially when the dressing procedure is carried out. To prevent these reflex movements, electrical stimulation is applied to contract the muscles, so that the carcass remains rigid. The electrical stimulation normally lasts for 40 to 45 seconds and is termed 'immobilisation' throughout the thesis.

The impact of immobilisation parameters on meat quality are of interest. The process evaluation by Carne showed that under the traditional low frequency (15 Hz), low

voltage (100 V) immobilization, the pH values of an indicator muscle, *longissimus dorsi,* were mostly lower than pH 6 at chiller entry, and therefore posed a high risk of heat shortening. Carne recommended that Silver Fern Farms upgraded the existing immobilization setting (15 Hz) to a high frequency 400 Hz in beef to minimise rapid pH fall while the carcasses were still hot. Carne Technologies also recommended that Silver Fern Farms should install a Smart Stimulation system, which was claimed to control the rate of pH fall during chilling and predict ultimate pH (Simmons et al., 2006).

Silver Fern Farms installed Smart Stimulation units in the Pareora lamb/sheep processing plant in 2008 and another in the Finegand (Balclutha) beef plant following the process evaluation. However, the outcomes were unknown and that is the partly the subject of this thesis. Because of the higher potential value for beef, my research was directed at Finegand only. The research aimed to evaluate the effectiveness of these new stimulation systems – high frequency immobilisation and Smart Stimulation – by measuring the subsequent meat quality and related events after slaughter to 21 days post mortem. This is because profound biochemical and structural changes are directly associated with the transformation of muscle tissue from the pre-rigor to post-rigor states. These transformations have crucial impact on the quality attributes and thus the choice of storage/processing conditions.

Chapter 2 will describe and discuss the biochemical reaction during the conversion from muscle to meat, the concepts of heat and cold shortening, and the role of electrical stimulation in improving meat quality with particular reference to gaps in knowledge. In respect of the new Smart Stimulation and high frequency immobilization system, it is noted that because this technology is very young, there is limited knowledge about its properties and no references in the meat science literature. This work will help to fill that gap.

Chapter 3 will describe the material and methods for the study, which includes the animal selection, processing treatment, measurements on muscles, data handling and statistical analysis.

Chapter 4 and **Chapter 5** reported the results and discussion for the two parts of this project, where bovine carcasses were subjected to three electrical treatments after

slaughter. Chapter 4 focused on the pH and temperature decline; whereas Chapter 5 discussed the meat quality attributes.

Chapter 6 concluded the main findings for the study and potential future work.

Chapter 2
Literature Review

Myology

Apart from the skin/hide, the carcass component of animals consists of three main parts: muscle, fat and bone. Of these, the most dominant is the muscle tissue. It comprises 30 to 40% of the live weight of an animal, which is about 100 to 150 kg of meat tissue (Lawrie & Ledward, 2006). The skeletal muscle tissue connects to bone and transmits force to bones to enable locomotion. Fat can be subcutaneous (sited under the skin of the animal), intermuscular (sited between individual muscles) or intramuscular (present within the body of a muscle). The subcutaneous fat is the most obvious and is easily removed to produce a leaner-looking meat (if that is what is wanted). The intermuscular fat is more difficult to remove and the intramuscular is an intrinsic part of the muscle (Warris, 2000). Where intramuscular fat levels are high, and thus obvious to the eye, the muscle is said to be marbled.

Under a transmission light or electron microscope, the skeletal muscle tissue is seen to be crossed by parallel lines (striations), and for this reason, the skeletal muscle is also called striated muscle. Its contractions are controlled by the higher nervous system, and thus can also be called voluntary muscle. Involuntary muscle – also known as smooth muscle because it is unstriated – is dominant in the viscera components such as intestines and glands, but is also present in artery walls (Lawrie & Ledward, 2006), but these are a minor part of carcass muscle and are of no interest in this study.

Individual skeletal muscles vary greatly in size and shape. In all cases however, they are made up of parallel arrangements of elongated, multinucleated cells called myofibres or muscle fibres. Each bovine myofibre ranges from 10 to 100 µm in width and from a few millimetres to several centimetres long, and sometimes spanning the entire length of a muscle. The myofibres are arranged in a hierarchical fashion, as shown in Figure 1. Each myofibre is encased in a layer of connective tissue – dominated by collagen – called the endomysium. On the next level, groups of myofibres are organised into primary and secondary fascicles that are separated by yet another layer of connective tissue called the perimysium. Finally, a layer of heavy connective tissue – the epimysium – surrounds the whole muscle. The endomysium

merges with the collagenous tendons that link the muscle to bones (Strasburg, Xiong, & Chiang, 2008).

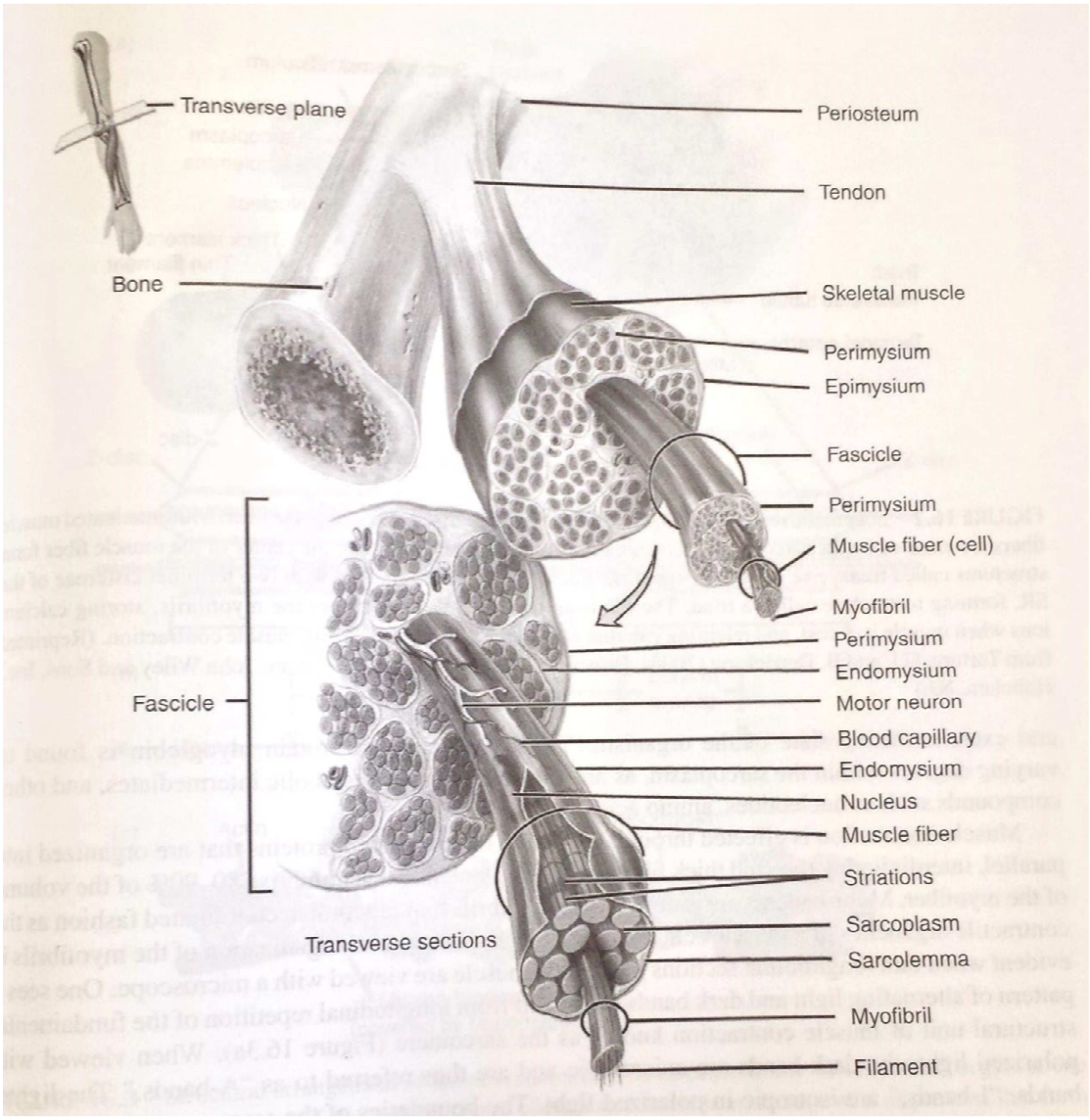

Figure 1 Diagrammatic representative of the structural organisation of muscle from subcellular myofibrils to whole organ (reprinted from Tortoa & Derrickson, 2006).

The muscle is permeated by a complex nervous system, which is responsible for the regulation of muscle contraction and maintenance of muscle tone, and a vascular system, in which the blood provides oxygen and nutrients to the muscle while also removing metabolic end products. The perimysium and endomysium combine to create the necessary framework that maintains the structural integrity of these tissues during muscle relaxation and contraction (Strasburg et al., 2008).

The structure of muscle cells allows the translation of electrochemical impulses into force. This is triggered by a neural stimulation, which then increases the intracellular calcium concentration and eventually triggers a muscle contraction, as explained below.

The myofibre is bounded by a plasma membrane, which is called the sarcolemma (Figure 2). The skeletal muscle sarcolemma differentiates from the plasmalemma of other cells by having periodic invaginations of the membrane into the interior of the muscle cell. The structure is like creating gaps by poking fingers into a plastic balloon skin. These invaginations of the sarcolemma - referred to as transverse tubules or T-tubules - transmit the depolarization signal for contraction from the neuromuscular junction to the interior of the myofibre. In the interior, the transverse tubules are physically contacted to the sarcoplasmic reticulum at periodic intervals. The Sarcoplasmic reticulum is an extensive and highly developed intracellular network that encircles the contractile organelles and acts as a reservoir of calcium ions, Ca^{2+}, which trigger muscle contraction after entering the sarcoplasm. Many proteins are embedded in the sarcoplasmic reticulum and are responsible for specific functions related to calcium regulation. Some proteins in the sarcoplasmic reticulum lumen bind calcium ions when the muscle is at rest (Rossi & Dirksen, 2006). Other proteins form channels that open in response to the depolarization signal and allow the diffusion of calcium ions from within the lumen to the sarcoplasm, triggering a muscle contraction (Dulhunty et al., 2002).

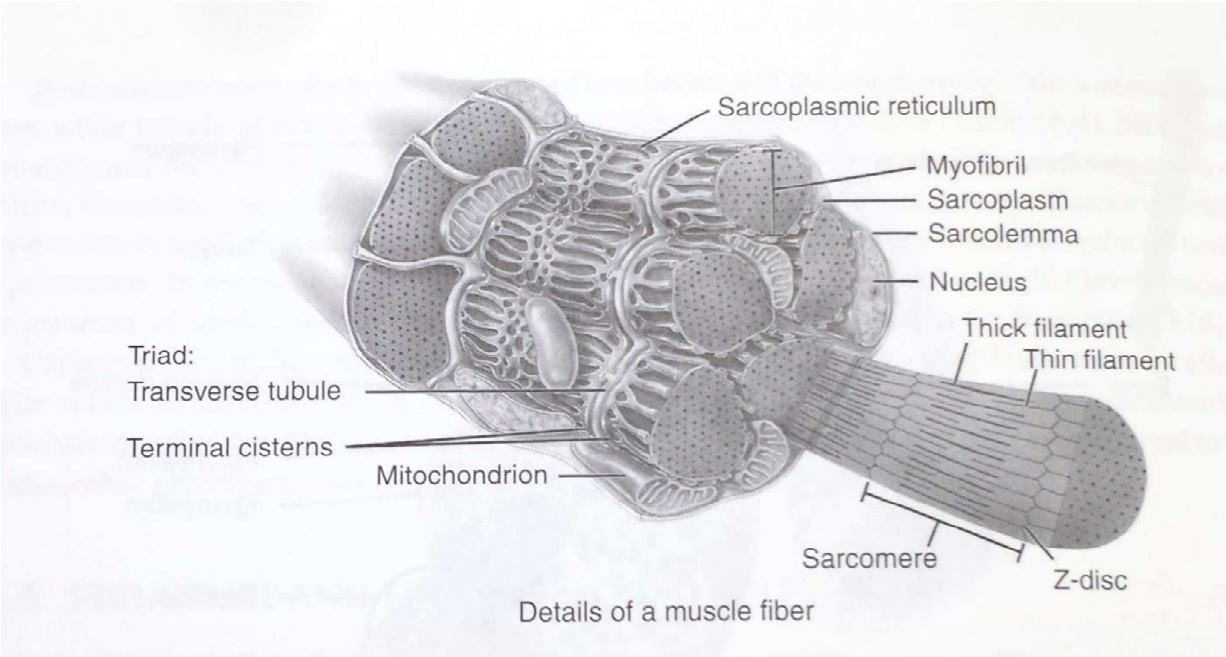

Figure 2 Schematic representative of the structural organization of a muscle fibre (reprinted from Tortoa & Derrickson, 2006).

The other muscle fibre features can also be seen in Figure 2. Specifically, the myofibres are multinucleated. The nuclei are distributed around the periphery of the cell and immediately beneath the sarcoplasmic reticulum. The mitochondria acts as energy transducers for the myofibre and is also found throughout the cell in close association with the contractile thick and thin filaments.

Muscle contraction is activated through the action of specialised proteins organized into the parallel, interdigitating thin and thick filaments (Figure 3a). The filaments make up 80 to 90% of the volume of a myofibre. Under polarised light microscopy, dark bands are anisotropic and therefore known as ‘A-bands’; whereas the lighter bands, ‘I-bands’ are isotropic. The boundaries of the sarcomere are defined as Z-discs, which are narrow, dark and electron-dense bands proteins in the centre of I-band. The protein matrix that comprises the Z-discs, acts as a foundation that supports the thin filaments that originate from both sides of Z-discs.

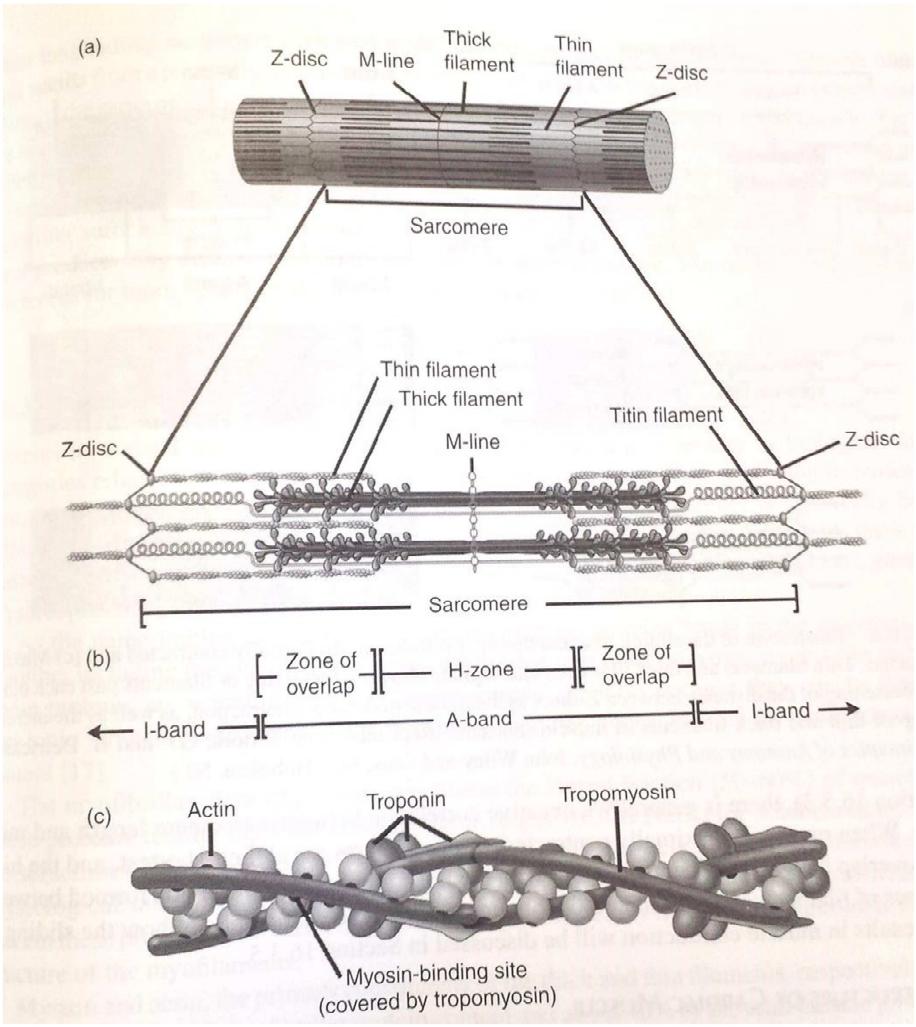

Figure 3 Structural arrangement of the sacromere (reprinted from Tortoa & Derrickson, 2006).

Figure 3b shows that the sarcomere is made up of alternately located thin and thick filaments. The I-band consists of thin filaments; whereas the A-band consists of overlapping thin and thick filaments. The centre of the A-band is slightly less electron-dense than the distal regions and thus appears brighter. This brighter zone is referred as the H-zone and consists of only thick filaments with no overlapping thin filaments. At the centre of H-zone, there is a dark zone analogous to the Z-disc: the M-line. It comprises proteins that maintain the structural arrangement of the thick filament protein and act as an anchoring point for the scaffolding protein titin (Strasburg et al., 2008). Figure 3c shows the structural detail of the thin filament,

where the dominant proteins are actin, tropomyosin[1] and troponin. Although not shown in Figure 3c, the sole protein of thick filament is myosin, to be discussed in more detail later.

Muscle contraction

Huxley and Hanson (1954) proposed a theory of muscle contraction named the sliding filament theory. This theory is based on the observations that the lengths of both thin and thick filaments remain constant, regardless of whether the muscle is stretched, contracted or in resting state. However, the sarcomere length – that is defined as the distance between the adjacent Z-discs – varies depending on the state of contraction or stretch force applied to the muscle fibre. When contraction takes place, the thin and thick filaments slide past each other and the sarcomere length becomes shorter. Conversely, during stretching, the thin filaments of sarcomere slide away from each other as they move along the A-band, the sarcomere length becomes longer (Figure 4).

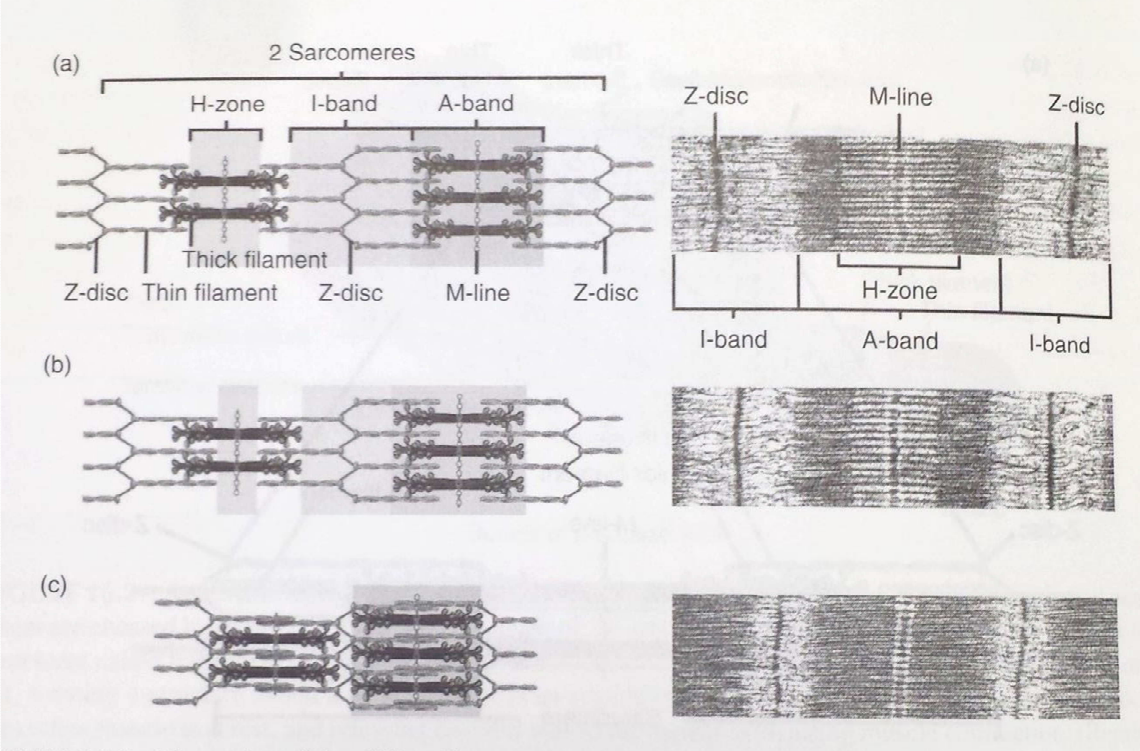

Figure 4 Illustration of the sliding filament theory: (a) Relaxed (b) Partially contracted, and (c) Maximally contracted (reprinted from Tortoa & Derrickson, 2006).

[1] Tropomyosin and troponin have subunits, but that is beyond the scope of this review.

From a meat science perspective, the extent of thin and thick filament overlap has a significant effect on meat tenderness (Huxley & Hanson, 1954). There is a negative correlation between the sarcomere length and meat toughness. When the muscle is maximally contracted, the sarcomeres are at their shortest, with the highest degree of overlap between the filaments and a large number of rigor bonds formed between the two filament types (Figure 4c). This results in increased toughness.

Molecular details of muscle contraction and relaxation

There is a significant difference between a live muscle tissue and meat. Once an animal dies, the muscle tissue enters a series of transitional reaction: the conversion of muscle to meat, which will be discussed in later section. The biochemical events are the same as muscle contraction in a live muscle, and are significant determinants of meat quality (Strasburg et al., 2008).

When a muscle is at rest, the voltage difference across the sarcolemma is about 90 mV and the intracellular Ca^{2+} concentration is very low: $<10^{-7}$ M. In the myofibrils, the tropomyosin is located on the actin filament in such way that prevents myosin-actin cross-bridge formation (the basis of muscle contraction). When a motor neuron that adheres to the sarcolemma (Figure 1) triggers stimulation, the muscle cell depolarizes at that neuromuscular join point. The depolarization propagates along the sarcolemma and the T-tubules. There is a mechanism in sarcoplasmic reticulum involved in this early phase of muscle contraction: the ryanodine receptor (RyR) and the dihydropyridine receptor (DHPR). The DHPR responds to the depolarization by undergoing a conformational change that is transmitted across the T-tubule to the RyR, which is located in the membrane of the sarcoplasmic reticulum terminal cisterns (Figure 2). The RyR is a channel that opens in response to the conformational change and allows calcium ions to flow into the sarcoplasmic reticulum. At this stage, the calcium concentration increases over 100-fold: from 10^{-7} M to $>10^{-5}$ M. The Ca^{2+} binds to the troponin complex (Figure 3c; Figure 5), causing a conformation change that is transmitted to tropomyosin. Consequently, the tropomyosin is pushed deeper into the actin groove (Figure 3c; Figure 5) exposing the actin filament to the myosin head, enabling actin-myosin cross-bridge formation (muscle contraction) (Figure 5) (MacIntosh, Gardiner, & McComas, 2005).

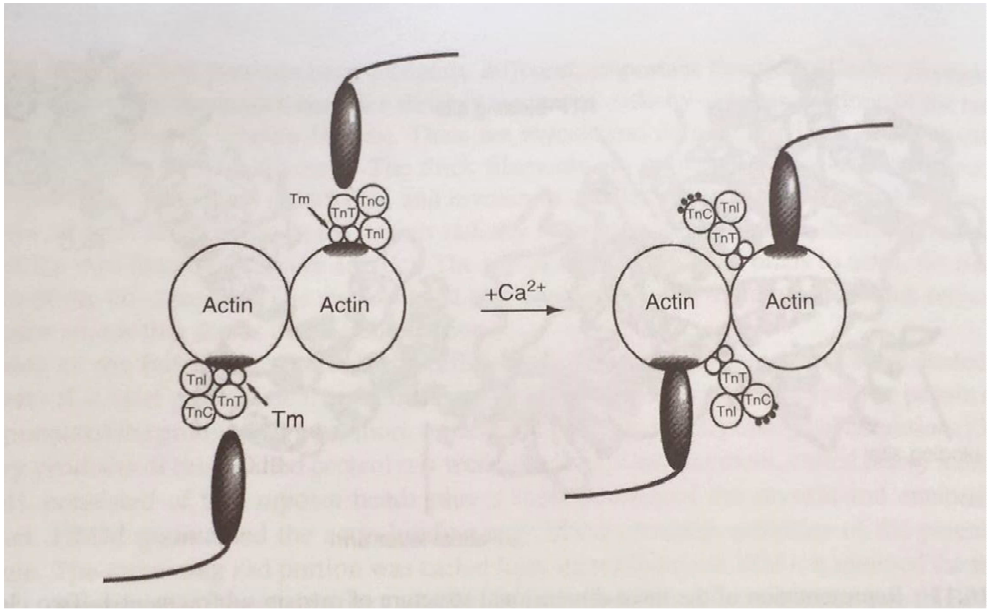

Figure 5 Regulation of contraction and relaxation by the tropomyosin-troponin complex. The dark lobes are myosin heads that can interact with the actin when the tropomyosin-troponin complex slides into the actin groove in response to Ca^{2+} binding (reprinted from Chiang, Strasburg, & Byrem, 2007).

A complete muscle contraction cycle is illustrated in Figure 6. The nucleotide-binding site of the myosin heads contains ADP and inorganic phosphate (P_i), which are the end-products from ATP hydrolysis (Figure 6 Step 1). The exposed myosin-binding sites on the actin filament allows a weak binding between the actin head to thin filament with an almost orthogonal binding to the thick filament axis. Upon binding, the myosin head releases P_i, causes a small conformational change, and strengthens the binding between actin and myosin (Step 2). This is immediately followed by the power stroke: a large conformation change where the myosin heads pull the actin filament along the thick filament towards the M-line, thus causing the sarcomere length to shorten (Step 3). ADP is released from the myosin head and the available ATP then binds to the empty nucleotide-binding site. This triggers myosin head to release from actin (Step 4). As long as the neuronal stimulation continues, which depletes ATP, the muscle contraction cycle will restart from Step 1 (Raymen & Holden, 1994).

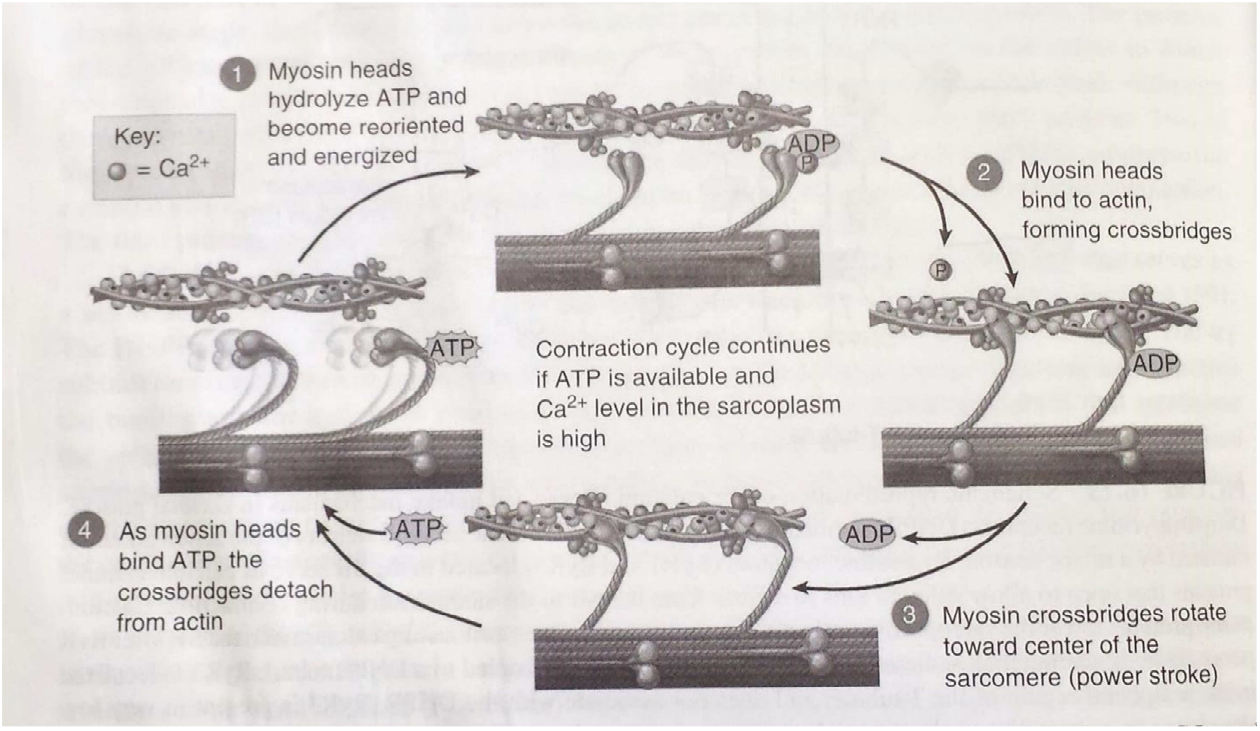

Figure 6 Cross-bridge cycling during muscle contraction (reprinted from Tortoa & Derrickson, 2006).

At the end of stimulus, the sarcoplasmic Ca^{2+} concentration lowers to its resting state through the activity of a calcium pump, which is present within the sarcoplasmic reticulum. The troponin complex thus becomes deficient in Ca^{2+} and the muscle reverts to a resting state, which blocks the myosin-actin cross-bridge formation (Strasburg et al., 2008).

To this point the muscle fibres have been considered as unvarying, and certainly the concepts described above apply to all skeletal muscles. However, muscle fibres show some variation even within a fibre bundle. Before discussing the conversion of muscle to meat after slaughter, it is useful to consider the broad variations in muscle fibre type: differences in the speed of contraction (fast versus slow) and supporting mechanism (oxidative versus anaerobic). Speed of contraction – termed twitch speed – can be measured in isolated muscle fibres held in a suitable physiological medium when a stimulus is applied.

Slow-twitch fibres are classed as Type I and fast-twitch are Type II. There are at least two subclasses of Type II: IIA and IIB (Young, 1984). Type I fibres that dominate slow twitch muscles – like the bovine masseter muscle of the cheek –

contain a high concentrations of mitochondria and lipids; and provide energy for oxidative metabolism. Since oxygen is needed for metabolism, slow twitch muscles are predominately red due to their myoglobin content. These muscles are involved in posture and steady repetitive actions: cud chewing or low speed swimming in fish; low speed swimming is accomplished by the subcutaneous lateral line muscle, which is notably red. Type II fibres (or fast twitch muscles) are more equipped for anaerobic metabolism fuelled by carbohydrate, for example during sprinting (Young, 1984; Spangenburg & Booth, 2003).

According to Young (1984), some carcass muscles are pure Type I: like masseter; and some pure Type II(B): like cutaneous trunci[2]. But most carcass muscles are a blend of the three basic types (Type I, Type IIA & IIB). Each muscle's composition reflects its role in posture, locomotion or repetitive action in the live animal, and in turn the composition will have some effect during the conversion of muscle to meat, which takes place during the 24 hours of so after slaughter.

Conversion of muscle to meat

As discussed in previous section, the muscle contraction requires a supply of ATP that contains a high energy phosphate bond (Figure 6). ATP is generated in the muscle cell by a number of mechanisms that result in a resting concentration of around 5mM. After the animal dies, blood cannot deliver oxygen and energy (carbohydrates and fatty acids) to muscle tissue; and remove carbon dioxide. Anaerobic glycolysis rapidly dominates the metabolic pathway to regenerate ATP lost by metabolism including the activity of the calcium pump. The substrates for anaerobic glycolysis are creatine phosphate and later glycogen, also known as animal starch. When creatine phosphate is exhausted, glycogen takes over. The enzyme phosphorylase cleaves monomeric glucose units from glycogen, yielding glucose-1-phoshate; and enters glycolytic pathway, generating limited quantities of ATP. Under the anaerobic environment, the end product of glycolysis is lactic acid (because the pyruvate cannot enter the Krebs cycle) (Strasburg et al., 2008). Since the bloodstream cannot remove lactic acid, the muscle will acidify and convert to meat. When the muscle glycogen is exhausted or the pH falls to levels where

[2] Cutaneus trunci is a pale muscle beneath the lateral trunk skin, and in bovines can be seen to twitch rapidly to discourage flies.

enzyme(s) activities become limiting, glycolysis ceases and ATP can no longer be generated. The myosin heads will permanently attach to the actin (lower drawing Figure 6) and the muscle is said to be in rigor mortis and is thus meat.

When the meat pH falls no further, it is called the ultimate pH (often abbreviated to pHu). The time to attain ultimate pH is variable and it is dependent on species, animal genetics, proportions of Type I, IIA and IIB fibres in a muscle, ante-mortem nutritional and animal management conditions that affect glycogen concentration at slaughter, and also processing treatments like electrical stimulation (Lawrie & Ledward, 2006).

An example of pHu attainment for different types of muscles is shown in Figure 7 (Bendall, 1978). Without any processing intervention, the process normally takes 4 to 8 hours for pigs, 12 to 24 hours for sheep and 15 to 36 hours for cattle (Figure 8) (Dransfield, 1994).

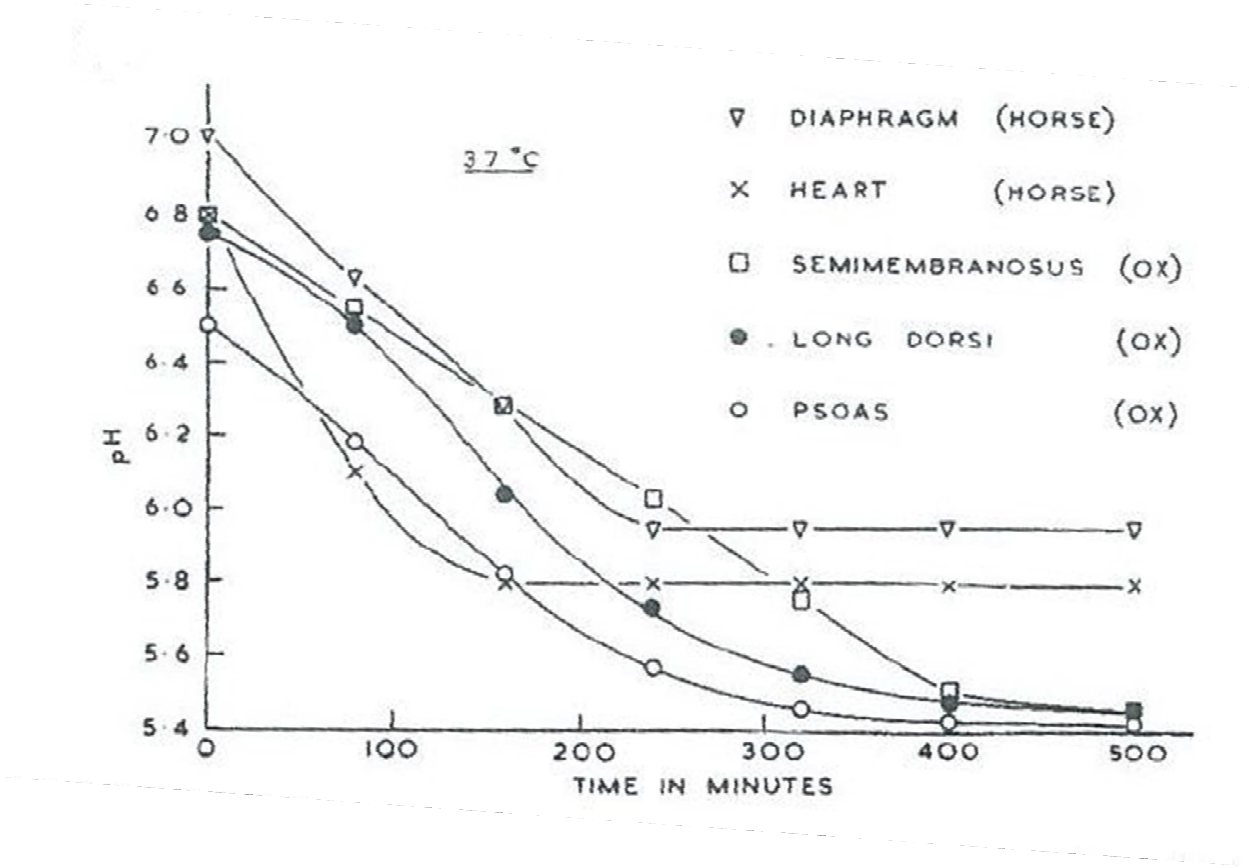

Figure 7 The effect of muscle type and species on the rate of post-mortem pH fall at 37℃. Zero time is this case was 60 min post mortem. The graphic is from Bendall, 1978)

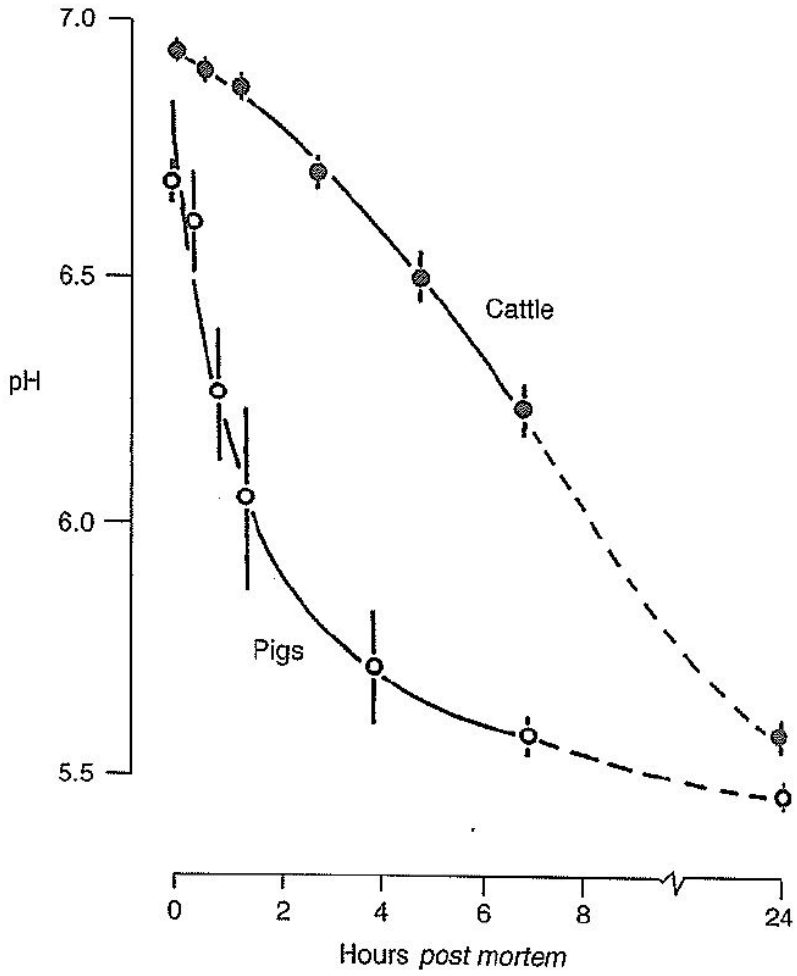

Figure 8 The rate of pH decline of the longissimus dorsi muscle in pigs and cattle. The curves are the average of 15 cattle and 30 pigs, with vertical bars as the standard errors (Dransfield, 1994).

Tenderisation of meat after rigor onset

It is well known that many higher priced chilled meat cuts are low in connective tissue. For these cuts, the cooked meat tenderness will improve during the days and weeks after slaughter. However, there is a financial cost associated with storing meat at all points of the value chain and after consumer purchase. Thus, there is a pressure to consume meat as soon as possible after slaughter, but at the cost of frequently tough meat. Also, there is another driver of early consumption: meat spoilage. The longer the meat is held and the warmer it is, the greater the chance of meat spoilage due to microbial growth. For these reasons, much research effort has been directed to understand the kinetics of tenderness development. Indeed, that is part of this research project that will be described later.

Tenderness development is influenced by endogenous proteases, which act on specific muscle proteins (but notably not collagen, which is why collagenous cuts can only be tenderised by extended cooking). At least two enzyme classes are involved: the calpains and cathepsins. The calpains have been studied intensively for their role in degrading post-mortem muscle protein and high activity in the neutral pH range. Calpains, of which there are at least two well-described variants, are calcium-activated enzymes that occur widely in nature. Their activities are regulated by calcium concentration, phospholipids and calpastatin. Calpastatin is a calpain-specific protein inhibitor (Goll, Thompson, Li, Wei, & Cong, 2002). After slaughter, when the muscle is gradually entering rigor, the Ca^{2+} concentration increases in the muscle fibre and activates the calpain system, which eventually initiates muscle protein hydrolysis, leading to tenderness development. In contrast to the calcium-activated calpains, the cathepsins are activated by the acidic pH condition (due to lactic acid generation), which prevails as ultimate pH in the days and weeks after slaughter. Both proteases (calpains and cathepsins) are responsible for hydrolysing muscle proteins like troponin-T, titin, nebulin, C-protein, desmin, filamin, vinculin and synemin (Huff-Lonergan et al., 1996), which are either directly attached or associated with the Z-discs. This process will contribute to meat aging and tenderness improvement (Strasburg et al., 2008). In a commercial environment with an export focus, as is the case in New Zealand, meat storage that allows tenderness development (ageing) is achieved by holding the meat at -1.5℃, just above its freezing point. The low temperature minimises spoilage (Lawrie & Ledward, 2006) and also slows tenderness development, so weeks may pass before optimum tenderness develops. However, that is not a problem in the export industry because it can take up to six weeks for chilled meat to reach its destination.

Meat quality

The discussion in the previous section implies that the kinetics of tenderness development is affected only by the temperature during ageing. However, this is not the case. In the early 1960s, New Zealand lamb processing industry received complaints from the Europe and North America consumers about excessively tough meat. Yet, the lamb consumed in New Zealand from the same abattoirs was not tough. The difference could be traced back to post mortem handling (Marsh & Thompson, 1958). The meat for the local market was not frozen; rather it entered cooling rooms prior to distribution and sold at chilled temperatures. This allowed ageing and improved tenderness. In contrast to export meat, the carcasses were blast frozen immediately after dressing and before the onset of rigor. This had two implications: the ageing process could not occur; and when the meat was cooled below 10℃ while the pH was still higher than 6.0 (before the onset of rigor), a phenomenon called cold shortening occurred.

The degree to which (excised) pre-rigor muscles contract in response to low temperatures is shown in Figure 9, indicating that the pre-rigor muscle will shorten significantly when the temperature is below 10℃. C old shortening is where the muscles permanently contract in response to high concentrations of Ca^{2+} in the muscle fibre. This is because at cold temperatures, the calcium pump becomes less effective in lowering the muscle cell Ca^{2+} concentrations (Pearson & Young, 1989).

The outcome is tough cooked meat when muscles have been cold shortened.

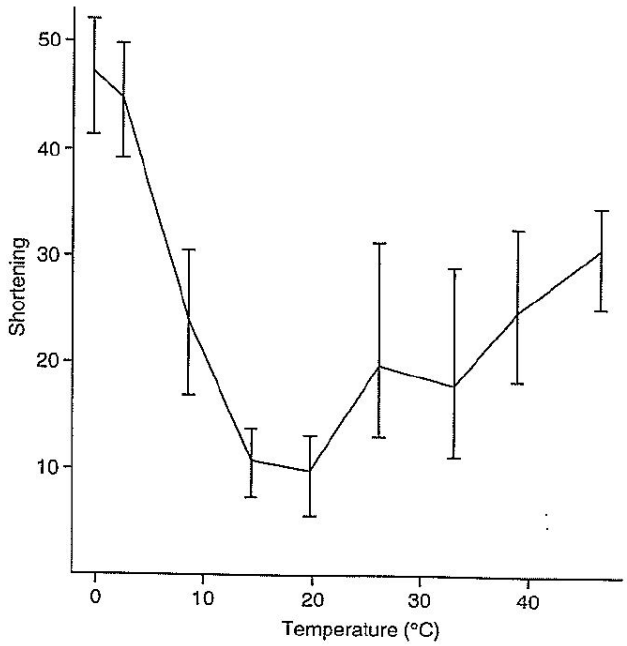

Figure 9 Percent shortening of excised pre-rigor beef muscle as a function of storage temperature (from Locker & Hagyard, 1963).

Figure 9 also shows another phenomenon: heat shortening. If muscles are held at temperatures above 30℃, while the pH falls below 6 .0 and before rigor onset, contraction will occur (Warris, 2000).

Geesink, Bekhit, & Bickerstaffe (2000) showed that a high pre-rigor temperature accelerates the attainment of low pH, which induces protein denaturation. Offer (1991) explained that because the contractile proteins are denatured at higher temperatures, combined with lower pH, the effectiveness of tenderising enzymes (calpains and cathepsins) is reduced. Protein denaturation also causes the myofibrillar proteins to shrink and expel fluid into the extracellular space. This manifests as excessive drip. Also, the shrinkage of the myofibrillar components increases the amount of light reflected from the meat. As a result, heat shortened meat is paler, less red and more yellow.

Thus, both high and low temperatures during rigor onset can adversely affect meat quality. The relationship between muscle pH and muscle temperature in Figure 10 shows how to avoid those conditions for the loin muscle (longissimus dorsi) in particular, and carcass muscles in general.

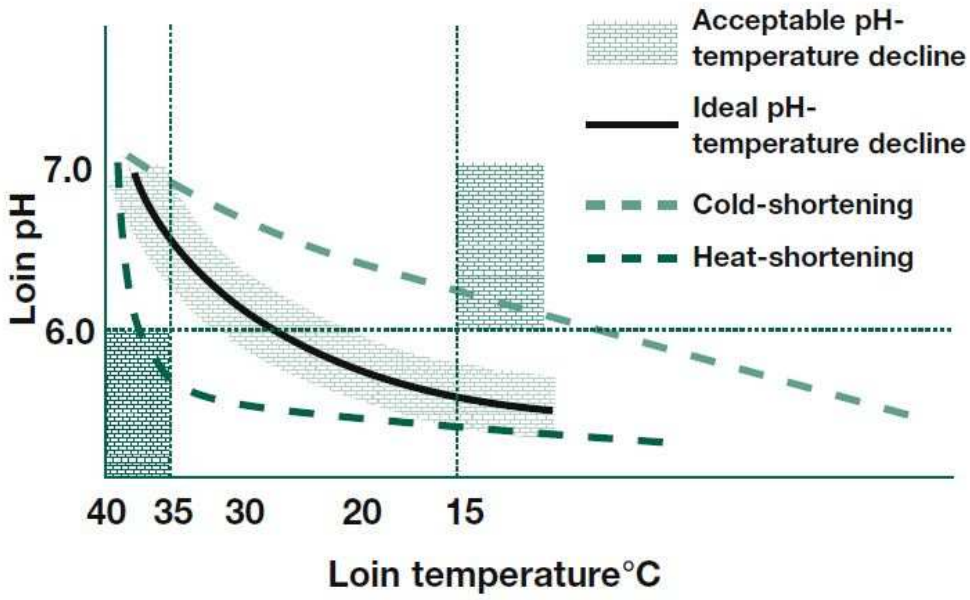

Figure 10 Post mortem pH-temperature plot showing the window through which muscle show pass to avoid cold and heat shortening (adapted from Meat & Livestock Australia, 2010).

Figure 10 suggests that cold and heat shortening could simply be avoided by controlling the rate of cooling and pH fall after slaughter. However, that is not a simple task because of different abattoirs having their own production speed, chiller capacity and electrical inputs to control rigor onset, the last of which is the focus of this thesis.

Electrical stimulation

History

In 1749, the polymath Benjamin Franklin discovered that an electric current applied across a turkey carcass immediately post-mortem would rapidly tenderise the meat (Lopez & Herbert, 1975). Years later, Harsham and Deatherage (1951) used high voltage electrical stimulation on freshly slaughtered beef carcasses to rapidly tenderise meat and filed a patent for the application. Commercial application was not seriously considered until 1973, when the first practical system was developed in New Zealand and then Australia to avoid toughness resulting from cold shortening. In New Zealand, electrical stimulation was initially used to accelerate rigor mortis before freezing sheep and beef carcasses, and now it is widely used to improve meat quality. Electrical stimulation is now considered as part of a total process from slaughter through chilling and optional freezing to final sale. For electrical stimulation to be useful, the waveform and pulse frequency, the stimulation duration, and the delay from moment of slaughter, must be matched to the species in question and the chilling rate. Table 1 summarises a number of typical electrical stimulation parameters in commercial use (Devine, Hopkins, Hwang, Ferguson, & Richards, 2004).

Table 1 Typical electrical stimulation parameters in commercial use.

Voltage (V)	Duration (seconds)	Frequency (Hz)	Delay from slaughter (min)	Country	Species
200	60	60	Immediate	U.S.A.	Bovine
1,130	90	14.3, alternate	30	N.Z.	Ovine
80	15-30	15, unipolar, square or half sine wave	5	N.Z.	Bovine
40	40-50	15, unipolar	5	Australia	Bovine

Beef electrical stimulation systems

Electrical stimulation can be applied in many methods. In this context, we are only interested in the beef electrical stimulation systems.

The beef stimulation systems extend to both batch and continuous methods. The batch method is administrated during immobilisation. The beef carcasses are immobilised on the bleeding table using a short metal bar or rubbing bar electrode that contacts the hindquarter muscles (Figure 11). A second option is to attach a battery clamp to the lip, with the return current carried by a clip or probe that attached at either the anus; or the chain that is used to hoist the animals. The first configuration is more frequently used as it is mechanically controlled, safer to operate and able to match up with the production speed (1 beef carcass per minute). Continuous systems consist of a series of stationary electrodes rubbing against the moving carcasses, with or without hides on (Figure 12**)** (Devine et al., 2004).

Figure 11 Batch method of stimulation applied with a rubber bar electrode.

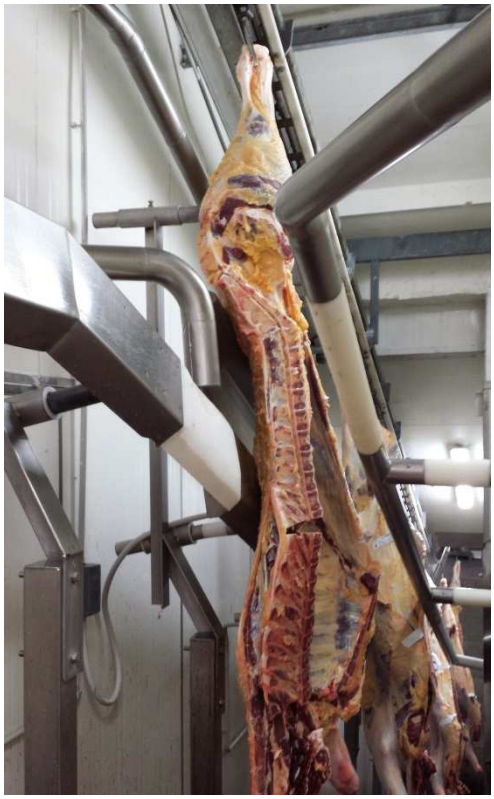

Figure 12 A continuous stimulation system.

Events during electrical stimulation

Electrical stimulation passes electric current through the muscles and causes vigorous muscle contractions. As a result, the muscle releases cathpetic enzymes that were associated with early tenderisation. Many researchers like Gilbert, Davey, and Newton (1977), Koohmaraie, Babiker, Merkel and Dutson (1988) and Kastner et al., (1993) agree that the electric current will affect the charge polarisation of the cellular membrane. The sarcoplasmic reticulum releases calcium ions into the myofibre and triggers contraction. This induced contraction enhances hydrolysis of ATP, which leads to accelerated glycolysis to regenerate ATP. Lactic acid is produced at the same time, so pH drops falls more rapidly, and rigor onset occurs much earlier.

For an industry that is focused on freezing meat as soon as possible after slaughter, electrical stimulation was quickly adopted to avoid cold shortening (Carse, 1973). However, the frozen meat could still be tough on subsequent cooking, because the meat had no chance to tenderise to optimum tenderness. Nonetheless, rapid rigor onset due to stimulation can trigger some tenderisation before freezing. According to Smith (1985), when electrical stimulation is applied immediately after the death of animals, Ca^{2+} ions are released at a time when the muscle temperature and pH are relatively high. This leads to early activation of calpains that results in greater proteolytic breakdown and gives tenderisation a head start. A stimulated carcass will attain ultimate tenderness much earlier time than unstimulated carcass (Figure 13).

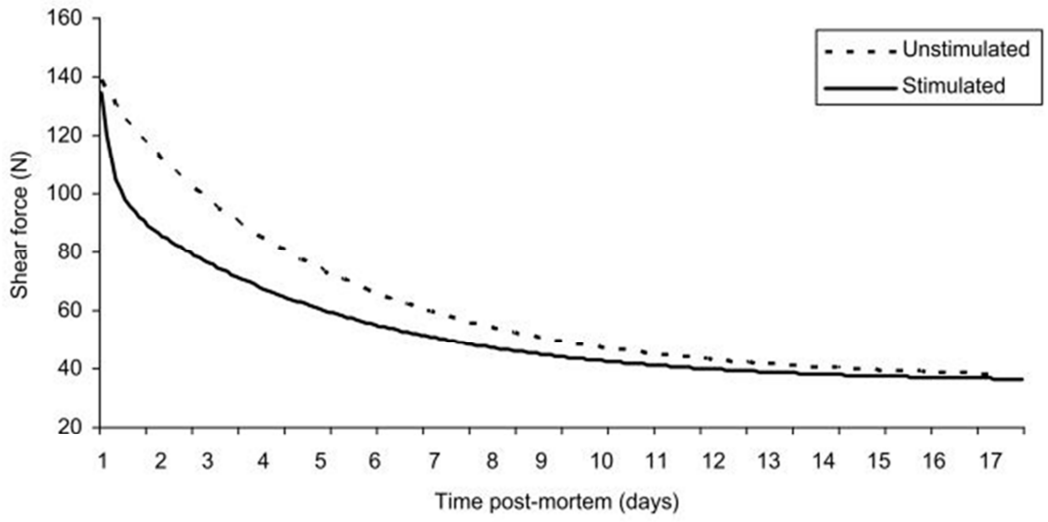

Figure 13 Tenderness curves for stimulated and unstimulated carcasses.

Although electrical stimulation is undeniably useful, problems can arise, one to be discussed now and others to be discussed in later sections. The most obvious problem is the potential for heat shortening. With accelerated glycolysis from electrical stimulation, the low pH meat can be exposed to a relatively high temperature (Figure 10). The rapid pH decline leads to some protein denaturation and greater reflectance of light. As a result, the colour of stimulated beef carcasses is brighter (Warris, 2000).

The paragraph above also suggests that electrical stimulation can be used to resolve 'heat ring' issues. The term 'heat ring' is defined as the two-tone appearance of the *longissimus dorsi*. With very cold air temperature at the beginning of chilling cycle, glycolysis is arrested on the outer portion of pre-rigor *longissimus dorsi* while pH is high, resulting in darker colour. In contrast, the inner portion of the muscle is unexposed to the cold air, chills at a slower rate and is subject to denaturation. The application of electrical stimulation will allow both muscles to decline at a similar pH rate.

What are the effects of electrical stimulation on muscle structure? This was an important question because the contractions induced by electrical stimulation are particularly strong. The question has been answered by transmission electron microscopy (Figure 14), showing a clear physical disruption within the muscle fibres. Savell, Duston, Smith and Carpenter (1978) suggested that the electrical stimulation at 100 V, DC, could improve tenderness by means of physical disruption and formation of contracture bands, but not necessarily by preventing cold shortening. The light micrographs from Hwang & Thompson (2002) revealed contracture bands throughout the myofibres, stretched areas on either side of the contracture bands and some contracture bands along with physical disruption of the myofibirils on either side of the bands (Figure 14).

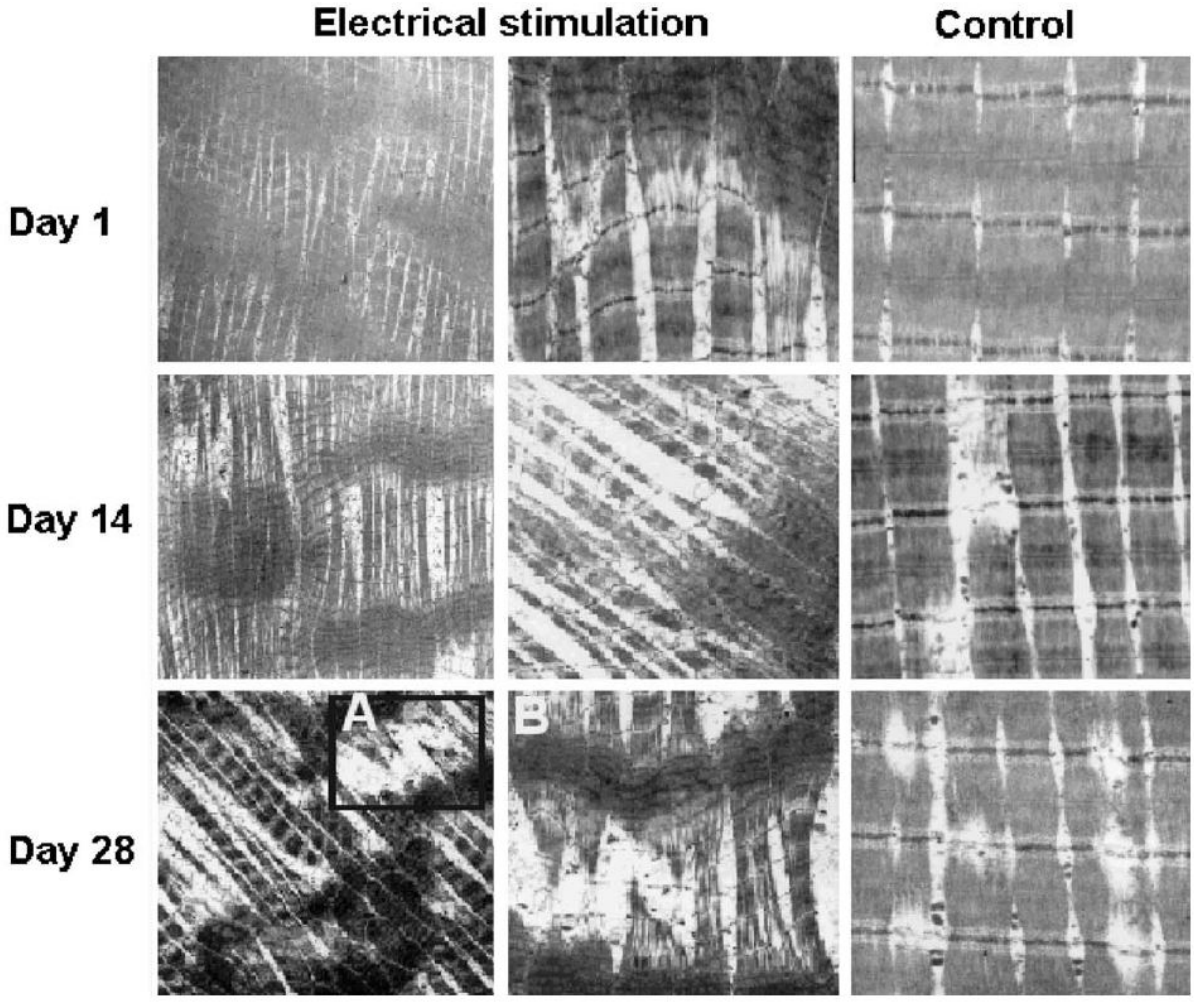

Figure 14 Characteristics of contracture bands in electrically stimulated beef longissimus compared with controls, and changes during ageing (reprinted from Hwang & Thompson, 2002).

In summary, electrical stimulation can elicit changes in post-mortem muscles by (1) acceleration of proteolysis; (2) prevention of cold shortening by ensuring rigor mortis is attained under optimum condition (pH and temperature); and (3) physical disruption of the muscle fibre (Hwang, Devine and Hopkins, 2003).

The descriptions and discussion above imply that electrical stimulation is responsible for the generally positive outcomes in meat quality. The electrical stimulation setting must be carefully tailored to prevent rapid decline of pH, or else heat shortening will occur that causes toughness and increases drip loss (Pearce et al., 2009). In our study, inappropriate stimulation setting could cause blood splash as well. The details will be discussed in later sections.

Before discussing the uncertainties and problems surrounding Silver Fern Farms' electrical stimulation technologies, it is useful to note the various electrical inputs that can be applied. Electrical inputs variously come from electrical stunning, immobilisation and any means of stimulation process that acts as a processing aid during or after the dressing procedure. Electrical stunning is used to either kill or render the animal unconscious prior to throat cutting. After death, the carcass is immobilised to allow for safe dressing procedures. A back stiffener is installed in some processing plants, which applies a current through the longissimus dorsi during hide pulling and causes muscle contraction (CSIRO, 2006). The combined strength of the contracted muscles and spinal column prevents damage/breakage to the muscle. With multiple electrical inputs that could be applied across the carcass muscle in a processing chain, they can contribute to pH decline rate and thus improve meat quality.

The pattern of pH decline is governed by the voltage, pulse amplitude, pulse width, frequency, muscle temperature and time, and the pre-stimulation time delay. These parameters will be discussed in details in the following sections.

Electrical stimulation types and factors affecting electrical stimulation

Voltage

Voltage determines the amount of electric current generated during electrical stimulation. It can range from 9 to 3,000 V and the effectiveness depends mainly on the animal species and the time of application after slaughter (pre-stimulation time delay) (Polidori, Kauffman & Valfre, 1996). Depending on the voltage, the electrical stimulation treatment is commonly grouped into: (1) extra low voltage stimulation, with a maximum peak voltage of 45 V; (2) low voltage stimulation, with a maximum peak voltage of 100 V and (3) high voltage electrical stimulation, ranging between 100 and 3,000 V (Bouton, Ford, Harris, & Shaw, 1980).

While the nervous system is still active post slaughter, a relatively low voltage electrical stimulation can be effectively applied (Devine, Hopkins, Hwang, Ferguson, & Richards, 2004). Polidori et al. (1996) recommended that low voltage stimulation is applied as soon as possible after slaughter. This is because it could stimulate through an active nervous system and give uniform effects throughout the muscles. When stimulation is delayed 30 to 45 minutes post slaughter and the nervous system is becoming inactive, higher voltage must be used to directly stimulate the muscles (Devine et al., 2004) . This theory is widely applied in many meat processing plants. Low voltage stimulation (<100 V) is deployed after neck cutting; whereas the high voltage stimulation is applied at the end of slaughter chain, typically using more than 400 V (Simmons et al., 2008).

In industrial practice, the lower the voltage, the less danger there is to the operator, and the safety requirements imposed by the electricity authorities will be less stringent for protecting the operator from electric shocks.

Pulse width and amplitude

The impact of pulse width and amplitude on muscle responses is based on the physiological principle of muscle contraction response. According to Aston (1991), for a given pulse amplitude, a reduction in the pulse width will simultaneously reduce the muscle response. However, the pulse amplitude and pulse width are interchangeable: the response that is lost by reducing the pulse amplitude can be regained by increasing the pulse width. These principles can be described in a

classic pulse strength-duration curve (Figure 15), which shows the threshold for producing an electrically induced stimulus.

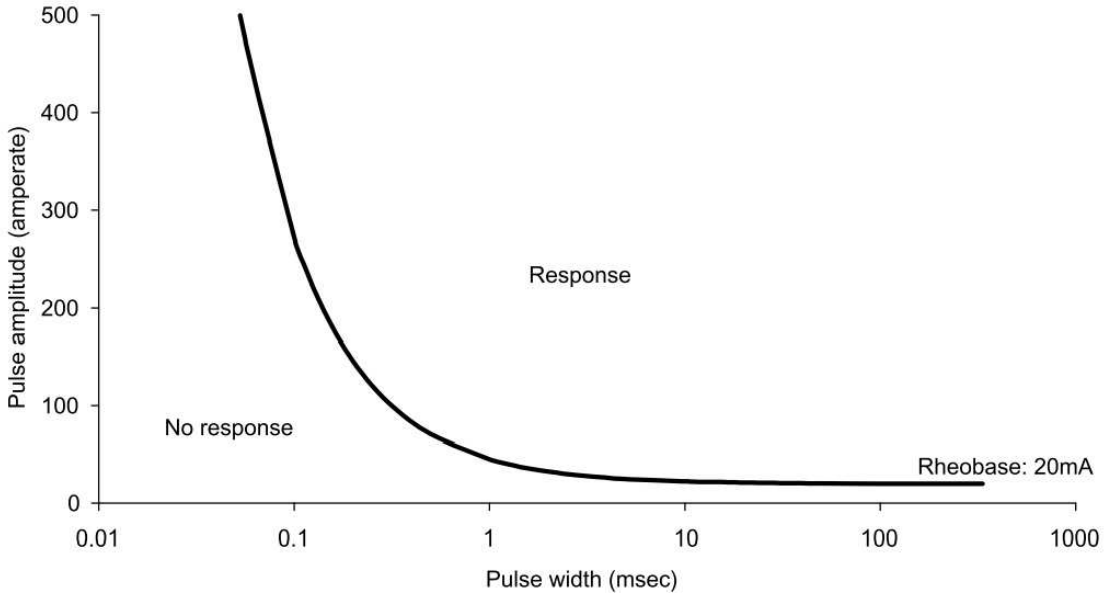

Figure 15 Pulse strength-duration curve to define the threshold for a response to an electrical stimulus (Aston, 1991).

Simmons et al. (2008) investigated the relationship between electrical stimulation parameters and muscle responses in lamb carcasses. They used a constant current stimulation unit that delivered electrical pulses of defined amperage and pulse width to stimulate the *longissimus* in lamb carcasses. The magnitude of the responses was measured by the intramuscular pressure changes.

The time of stimulation application after slaughter determined the effectiveness of stimulation. Simmons et al. (2008) found a distinctive pattern when the carcasses were stimulated immediately after slaughter: the threshold current required to produce a muscle response could be less than 50 mA. The muscle pressure curve (Figure 16) showed a very steep increase and attained a maximum muscle pressure after 10 seconds of stimulation. Then, after a further 30 seconds of continuous stimulation, the muscle pressure was reduced by 80%. They concluded that the muscle contraction responses are highly mediated by nervous system at this time.

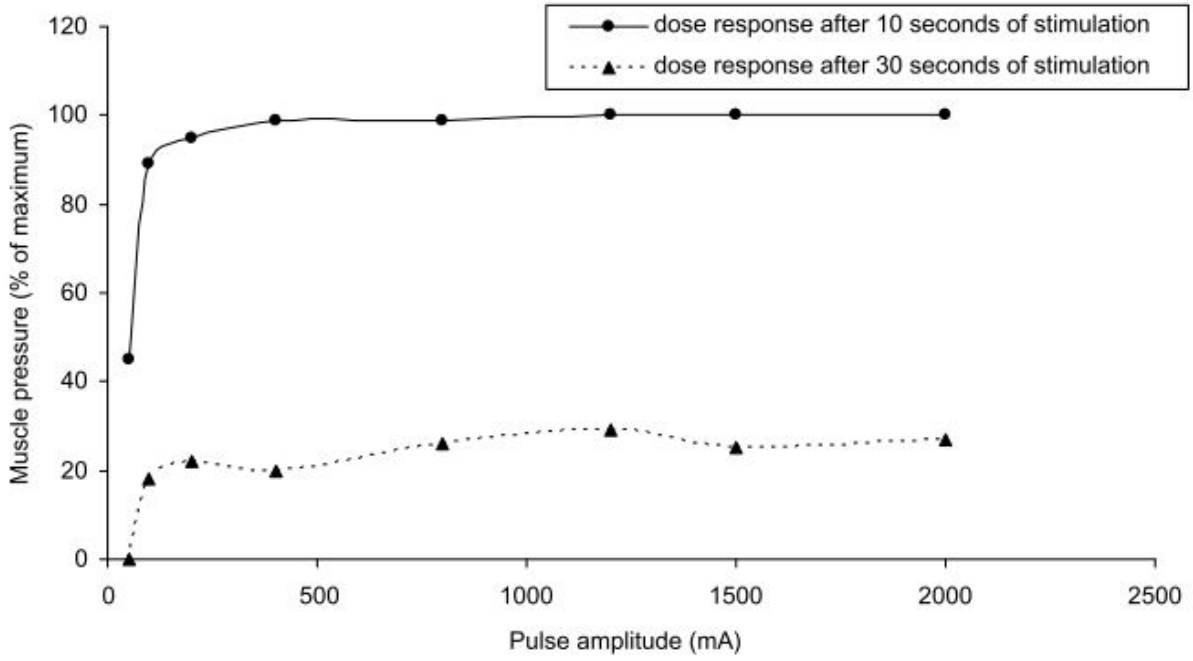

Figure 16 Muscle pressure response of a lamb carcass to electrical stimuli immediately after slaughter, 10 seconds of stimulation, and then followed by 30 seconds of stimulation.

Another trial was carried out by Simmons et al., 2008 on lamb carcasses at 20 to 30 minutes post slaughter. The carcasses were stimulated for 10 seconds, and then 30 seconds – both with 0.5 msec pulse width. The same cycle was repeated with a 10 msec pulse width. Figure 17 shows that as the carcass becomes increasingly fatigued and the pH declined, higher pulse amplitude and wider pulse widths are required to maintain the muscle response.

Thus, by applying all the above information/knowledge, it is possible to tailor and optimise a stimulation system to satisfy a particular industrial outcome.

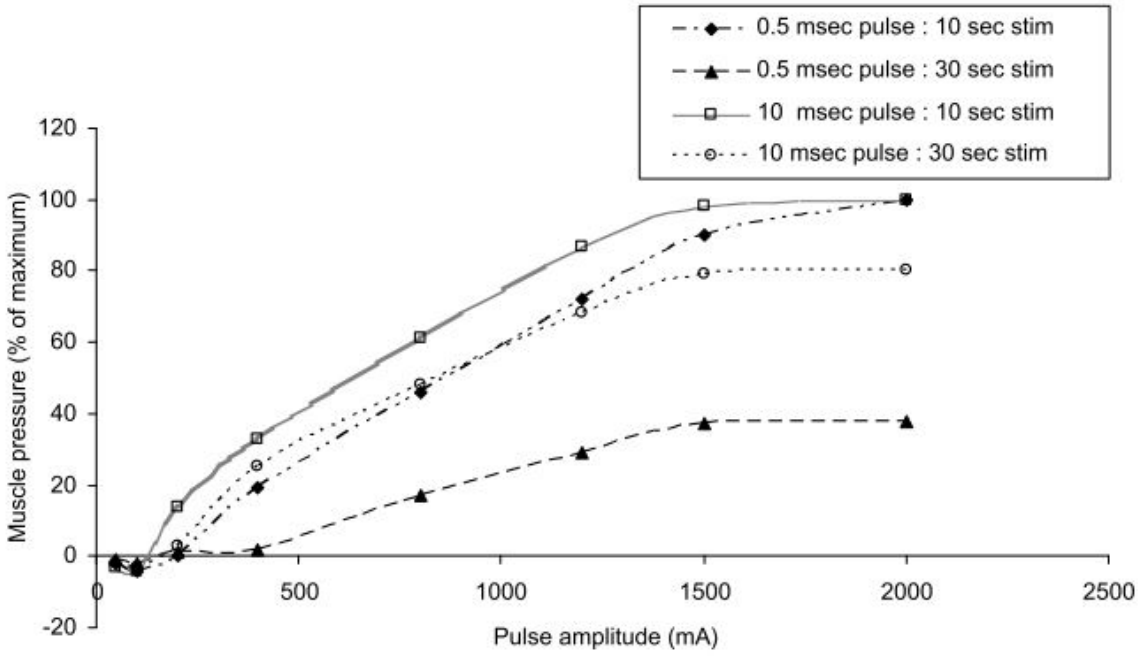

Figure 17 Muscle pressure response in a lamb carcass at 20 to 30 minutes after slaughter: Comparison of responses to two different pulse width under 2 different times: after 10 seconds, then followed by 30 seconds of stimulation.

Frequency

Takahashi, Lochnert, & Marsh (1984) and Takahashi, Wang, Lochner, & Marsh (1987) evaluated how different frequencies relate to the contracture bands formation in muscle (Figure 14) and to tenderness improvement. These studies showed that the electrical stimulation of 50 to 60 Hz at 500 V, 40 minutes after slaughter, would create excessive structural disruption and improve tenderness; a 2 Hz treatment at 40 minutes after slaughter did not affect structure and failed to improve tenderness. They concluded that the formation of contracture bands depends on the frequency. With a 2 Hz frequency, the time interval between successive stimuli is at 0.25 seconds. That allows ample time for muscle relaxation and therefore no cumulative effect to provoke a tetanic response. Thus, the muscle tetanic shortening is reversible, and the muscle can return to its original length after stimulation. However, at higher frequencies, there is insufficient time for relaxation between the twitches. Eventually, the muscle achieves supraphysiological tetanus and forms irreversible contracture bands (Figure 14).

Simmons et al. (2008) evaluated the relationship between frequency and the fraction of maximal muscle contraction on ovine *longissimus* at 15 and 50 Hz (Figure 18). The higher frequency produced a higher peak of muscle contraction force, but the response decay was much faster. The areas under the curves, which reflect the rate of pH decline, show that the pH decline rate at 50 Hz is about 35% less than at 15 Hz. These results were similar to those found by Chrystall and Devine (1978), where the maximum pH differences due to stimulation – 0.7 pH units – was achieved from 5 to 16.6 Hz; declining to 0.60 pH units at 25 Hz and to 0.50 pH units at 100 Hz (Figure 19).

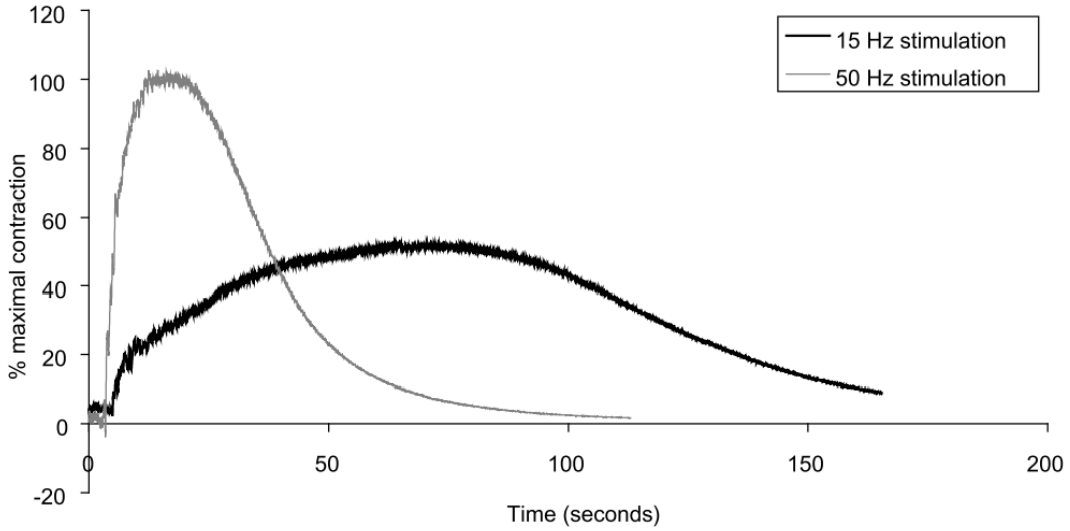

Figure 18 Muscle pressure response in lamb carcasses stimulated at 15 and 50 Hz (adapted from Simmons et al., 2008)

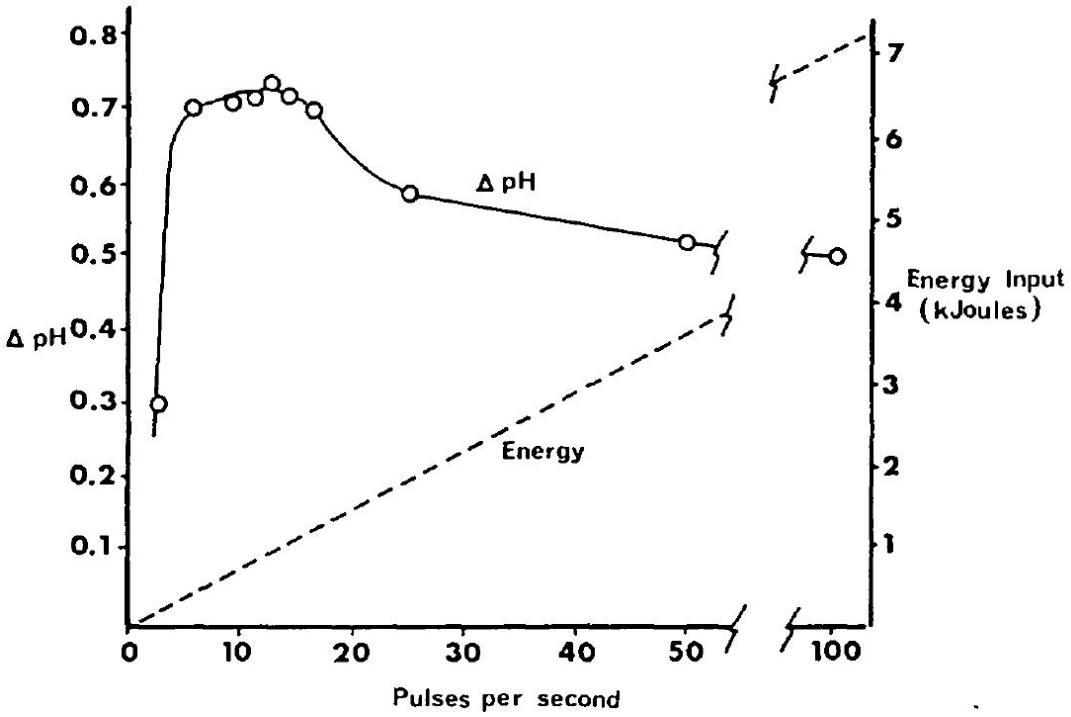

Figure 19 Effect of frequency on ΔpH for 120 seconds (adapted from Chrystall & Devine, 1978).

Muscle type and temperature

The effect of electrical stimulation is also dependent on fibre type. Muscles with a higher fraction of slow-twitch-oxidative (Type I) fibres (e.g. *longissimus dorsi*) respond less intensively to electrical stimulation than muscles with a higher percentage of fast-twitch oxidative-glycolytic (Type IIA) or fast-twitch-glycolytic (Type IIB) fibres (e.g. *semimembranosus*) (Devine, Ellery, & Averill, 1984).

When muscle temperature decreases, the magnitude of the pH fall reduces. For example in beef *sternomandibularis*, the pH change due to stimulation was 0.6 pH units at 35℃, but only 0.018 pH units at 15℃, sta rting from the same initial pH (Devine et al., 2004).

Meat quality outcomes of electrical stimulation for Silver Fern Farms

The quality outcomes of electrical stimulation are usually positive. However, Carne Technology's process evaluation for Silver Fern Farms showed that beef carcasses have routinely been overstimulated thus posing a risk of heat shortening. To overcome this problem Carne Technologies introduced high frequency immobilisation and Smart Stimulation to the Finegand beef processing plant.

High frequency immobilisation

Simmons et al. (2006) performed trials on sheep carcasses and demonstrated that high frequency electrical immobilisation – up to 400 Hz and medium voltage – had minimal effect on pH fall. Based on this result, the proposal was that with beef carcasses, the meat pH drop should be minimal before and after high frequency immobilisation. That would create a condition where the subsequent pH decline rate could be controlled and standardised by Smart Stimulation.

Smart Stimulation

The Smart Stimulation technology records the contraction responses of carcass sides as they are progressively stimulated, in such a way that when a desired pH is reached in an indicator muscle, chosen to be the large and valuable *longissimus thoracicus et lumborum*, stimulation stops (Simmons et al., 2006). It is one of the three stimulation systems compared in this thesis. The Smart Stimulation unit can be configured in two ways, either a small stimulation bar unit or stimulation tunnel. Figure 20 shows a small Smart Stimulation unit installed in the Finegand abattoir, where the stimulation is applied 30 minutes after slaughter. When a carcass reached the test weight sensor on the rail, the stimulation bar contacts and stimulates the *longissimus thoracicus et lumborum* muscles. This is where the study is carried out.

Figure 20 A Smart Stimulation unit in the Finegand abattoir, where the stimulation bar contacts the *longissimus thoracicus et lumborum* muscles.

Meat quality outcomes

Under Carne's recommendation, Silver Fern Farms upgraded their immobilisation settings to high frequency 400 Hz in all beef processing plant. However, soon after the upgrade, the company identified two major quality issues: burst tenderloins (Figure 21) and blood splash, also known as ecchymosis (Figure 22).

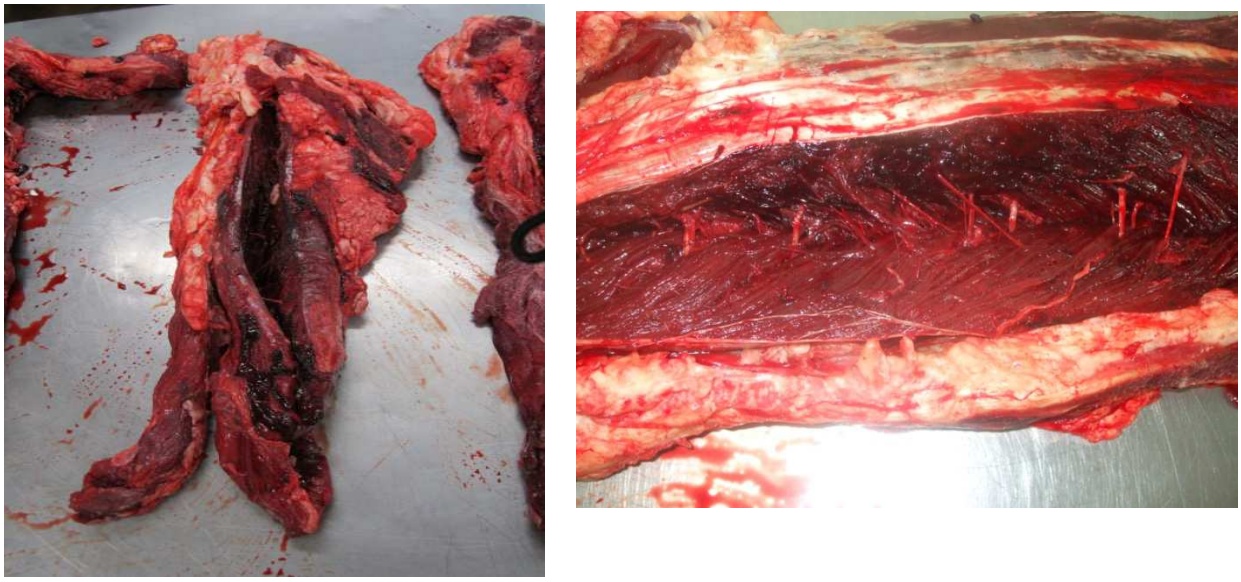

Figure 21 Burst *psoas major.*

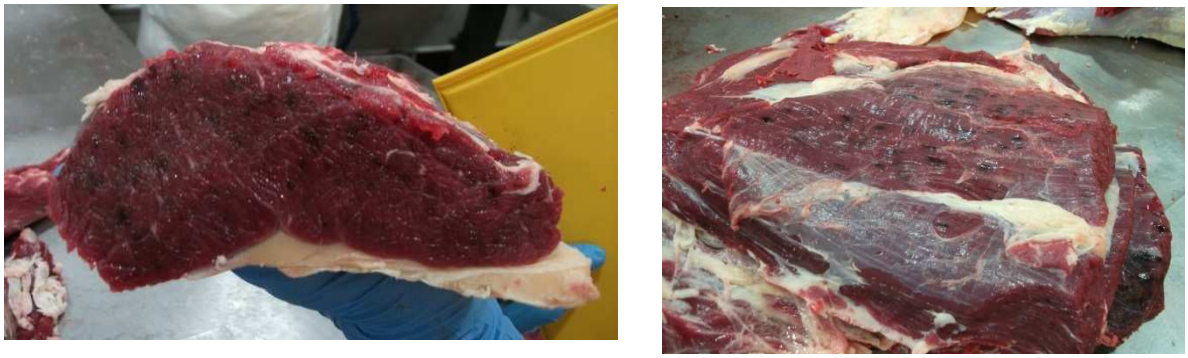

Figure 22 Blood splash on striploins (*longissimus dorsi*) (right) and on chuck (*biceps femoris*) (left).

With these unexpected problems, I spent several months collecting muscle information in all processing plants and held many discussions with Carne Technologies. Together we aimed to come up with an optimum immobilization setting. Ecchymosis is normally caused by a momentary and rapid rise in blood pressure at the time of stunning, leading to the rupture of capillaries. The issue becomes worse when there is a delay between stunning and bleeding (Collins, 1954). The company has reviewed its stunning and slaughter procedures and failed to find any outstanding change, apart from the upgrade in immobilization setting. Thus, we proposed the following theory.

Figure 18 demonstrates that with high frequency immobilisation, the muscle attains maximal contraction pressure within a very short time. In our protocol, the high frequency immobilisation - up to 400Hz - is applied immediately after slaughter. We speculate that the stimulation increases the blood pressure momentarily and the contraction force exceeds the limit that the muscle structure can withstand. This would explain why we observe blood clots and broken muscle fibre inside burst tenderloin cuts (Figure 21).

The unsatisfactory meat qualities from the 400 Hz immobilisation have resulted in a change to the protocol. From Carne's recommendation, the immobilisation setting has changed from continuous 400 Hz to an alternating mixture of 15Hz and 400 Hz, identical to a protocol used by Pearce et al. (2009) on sheep carcasses. This mixture is intended to initiate some muscle contraction pressure at 15 Hz before attaining maximal muscle pressure with 400 Hz. An analogy is warm-up exercises prior to a 100 m sprint race, so that you would not suffer from muscle cramp or injury.

Since 15 Hz accelerates pH decline rate (Figure 19) and 400 Hz could damage muscle, the frequencies setting pattern is important. After several optimisation trials, we settled on a frequency pattern that had minimal effect on pH decline and reduced blood splash and burst tenderloin incidence.

Objectives of the thesis

The new setting replaced the traditional 15 Hz immobilisation setting in all processing plants. However, the impact of the new setting on various meat quality parameters – pH decline rate, tenderness, drip loss, colour, etc. – was unknown, and how it differs from the traditional 15 Hz immobilisation was of interest. That is the first objective of this study.

We thus devised three treatments: the traditional 15 Hz treatment is Stimul 1 and the new setting, 15 Hz and 400 Hz. is Stimul 2. Stimul 3 is Stimul 2 paired with Smart Stimulation; the effect – if any – of Smart Stimulation on meat quality has yet to be demonstrated.

The second objective of this study is to understand the behaviour of representative meat cuts in response to the three stimulation treatments. The output from this work will be a comprehensive muscle profile database of commercial value. The intention

was to build a database through a monitoring of pH-temperature profiling, purge loss, colour, and tenderness development. This will allow Silver Fern Farms to recommend optimal ageing times before consumption or freezing.

Chapter 3 will describe the materials and methods for the study.

Chapter 3
Material and Methods

Animal selection

This experimental design study was conducted in a commercial abattoir at Finegand (ME26), Otago, in mid July 2013. The carcasses of 36 pasture-fed steers (male castrates), aged 18 to 28 months and weighing between 280 and 330 kg were singly selected for the study. The subcutaneous fat thickness was between 2 and 4 mm. The steers were electrically stunned across the head only, and bled according to Ministry for Primary Industries (MPI) approved humane slaughter requirements. The time at this point of the experiment was designated Day 0. After the electrical stimulation treatments, the carcasses were dressed according to standard New Zealand industry practices.

Electrical stimulation treatments

The 36 carcasses were randomly assigned to three stimulation groups. The stimulation settings and the number of animals in each treatment are listed in Table 2.The Phase I and II stimulation treatments were conducted during bleeding, which was immediately after the first stick on the throat. The treatment was applied by pushing an electric bar perpendicularly (Figure 11) against the hind and Achilles tendons of the animals for 40 seconds. Further down the production line, the new stimulation system, called the Smart Stimulation (Phase III), was applied approximately 30 minutes from exsanguination. The new system was applied by pushing a U shape stainless bar against the back of the carcasses, where the long, valuable *M. Longissimus lumborum dorsi*, otherwise known as the striploin (STRL), was located (Figure 20). Three stimulation cycles were programmed on Smart Stimulation. By recording the contraction responses of the *longissimus dorsi* (STRL) muscle, the stimulation cycles were different for each carcass in order to attain its desired pH at that time post slaughter.

Table 2 Electrical stimulation treatments

Stimulation	Phase I	Phase II	Phase III	Number of carcasses
1	5 ms pulse direct current, 100 V, 15 Hz, continuous for 40 seconds	None	None	12
2	5 ms pulse direct current, 100 V, 15 Hz, continuous for 4 seconds	0.3 ms pulse direct current, 2.5 ms interval, 100 V, 400 Hz, continuous for 36 seconds	None	12
3	5 ms pulse direct current, 100 V, 15 Hz, continuous for 4 seconds	0.3 ms pulse direct current, 2.5 ms interval, 100 V, 400 Hz, continuous for 36 seconds	0.5 ms pulse DC, 300 V, 15 Hz (Smart Stimulation)	12

Measurements on muscles

pH and temperature profiling with two indicator muscles

As it is extremely difficult to access and monitor all 6 muscles on the entire carcass, only two muscles were chosen for this work, i.e. the superficial *M. longissimus dorsi*, also known as the striploin; and the deeper *M. semimembranosus*, known as the topside. Intuitively, the deeper muscles will cool more slowly than superficial muscles, so the choice of these two was designed to give an indication of the range of cooling profiles that a carcass would encounter during rigor onset.

The aim of this work, which was done on up to 15 carcasses per stimulation treatment, was two-fold. Firstly, it would reveal between-animal variability for each treatment: the rationale being that low between-animal variability is desirable for consistent quality. Secondly, it would show if, on average, a given stimulation treatment would present risks of cold- and heat-shortening. The pH values for 15 carcasses on each stimulation treatment were reviewed on the basis that the latter pH value should be lower than the previous value during the 8 hour post slaughter period. The second selection criteria is based on the ultimate pH (pHu), carcasses with pHu>5.8 is excluded. This is because the study is targeted on high value chilled market products and the customers had specified the company to exclude high pH, dark meat carcasses. The 15 carcasses were filtered down to 12 carcasses and preceded for fabrication.

Six pH and temperature measurements were taken from each carcass with a TESTO® 205 pH meter (Testo AG, Lenzkirk, Germany) at approximately hourly intervals from the start of chilling to about 8 hours post slaughter, then finally at 24 hours: the pHu. The pH meter was calibrated before use and at regular intervals using pH 4 and pH 7 buffers at room temperature (Thermofisher, New Zealand). The glass probe was inserted through the *longissimus* between the 12th and 13th ribs and into the core of the deeper *M. semimembranosus* on the hindquarter.

The carcasses were spray chilled according to the programme shown in Table 3. After 20 hours of refrigeration, a final pHu was measured at the designated Day 1.

Table 3 Spray chilling programme.				
Cycle	1	2	3	4
Temperature (℃) set at	8 (6 to 10)	6	4 to 6	4 to 6
Hours set at	4	4	12	Infinite
Humidity (%) set at	98	98	98	98

Collection and storage of individual meat cuts

One day after slaughter (Day 1) six single muscles: *rectus femoris*, *longissimus dorsi*, *psoas major*, *gluteus medius*, *gluteobiceps* and *infraspinatus* were excised from one side of the carcasses by boning personnel in the abattoir. The muscles were checked against New Zealand beef cut specification (Beef and Lamb New Zealand, 2010)

Depending on the size and orientation of each muscle, 2 or 4 submuscles (100 mm x 50 mm x 50 mm) were excised, trimmed of visible fat, and labelled as Position 1, 2 etc. from a defined site depending on the muscle (Table 4). In summary, a grand total of 648 samples were collected in this study.

Table 4 Description of muscle subsamples

Muscle name	Abbreviation	Number of cuts	Position numbering direction
Longissimus dorsi	STRL	4	Anterior to posterior
Psoas major	TEND	2	Anterior to posterior
Rectus femoris	KNUCK	4	Dorsal to ventral
Gluteus medius	RUMH	2	Dorsal to ventral
Gluteobiceps	RUMC	2	Dorsal to ventral
Infraspinatus	BLD	4	Dorsal to ventral
Total cuts from each carcass		18	
Total cuts from each treatment		18 x 12 = 216	
Grand total from 3 treatments		216 x 3 = 648	

Each muscle subsample was vacuumed packed and randomly assigned to one of six different aging days, 1, 3, 5, 7, 14 or 21 days. As some muscles were small, it was not possible to assign subsamples to all six days. The muscles were stored and aged at -1.5 ± 2℃. The samples from the 5, 7, 14 and 21 d ay groups were shipped chilled to the Belfast, Canterbury laboratory. Temperature data loggers were randomly inserted at the thermal centres of each 22 kg cartons to monitor temperature fluctuation during transportation. Fluctuations were found to be minimal. At the prescribed day, the chilled muscles were measured for drip loss and colour, transferred to a plastic bag, and then blast frozen at -18℃ for at least two day s. The tenderness measurements were carried out as described later.

Drip loss

Drip loss was determined with a pan balance accurate to 10 mg. The meat was first weighed in its vacuum bag, then weighed after removal from the bag. The empty package was cleaned with warm water and dried overnight in a 30℃ incubator, then weighed. Drip loss was calculated by the following formula:

$$\text{Drip loss } \% = \frac{\text{Total weight of meat and packaging} - \text{meat weight} - \text{cleaned packaging x 100}}{\text{Meat weight}}$$

Colour

Thirty minutes after meat was removed from packaging and exposed to air, lean meat colour was recorded with a calibrated Hunter Lab MiniScan® XE plus spectrophotometer (Hunter Associates, Reston, USA), Model D45/0-s, using D65 light conditions, a 10°standard observer through a 5 mm aperture in the measuring head. Colour was recorded in CIE colour space for L*(lightness), a* (redness/greenness) and b*(yellowness/blueness). These values were further calculated for Hue angle and saturation index by the following formula (AMSA, 2001):

$$Hue\ angle = \tan^{-1}(b*/a*)$$

$$Saturation\ index = \sqrt[2]{a*^2 + b*^2}$$

Additionally, the L*, a * and b* values were converted to a reference value that is equivalent to colour tiles. This value has been commonly used in Japan as a way to measure meat colour. Also, the value serves as a commercially benefit communication tool between the company and its customer, as the colour can be physically visualised.

Figure 23 Japan beef colour standard score

Silver Fern Farms has conducted a number of calibration exercises for each Hunter meter. These exercises involved panellists who had been trained at scoring Japan meat colour tiles. Then, the results were correlated against L*, a* and b*, and derived a relation equation. For this trial, the relation equation for the Hunter meter used is:

$$Japan\ colour = L * \times (-0.22135) + a * \times (-0.001273) + b * \times (-0.008098) + 12.53046$$

Tenderness

The frozen subsamples were placed in weighted plastic barrier bags and cooked from frozen in a water bath maintained at 85 ± 2^{o}C. The water bath was fitted with a circulator and a Grant Optima™ GR150 thermostat/circulator (Grant, X, Y) (Figure 24). After 10 minutes, a T-type thermocouple was inserted to the approximate thermal centre of mass, which the temperature was displayed in a 3081 Hybrid Recorder (Yew, Yokogawa Hokushin Electric, Japan) (Figure 25). When each subsample reached 75 ± 2℃, the bagged cooked meat was remove d from the bath, the thermocouple withdrawn and the bagged meat was chilled in ice slurry for at least 30 minutes.

Subsequently, 10 × 10 mm cross section strips (4 to 6 replicates for each piece) were excised parallel to the fibre direction, which were sheared perpendicularly by the MIRINZ tenderometer (Figure 26). The results were displayed in kPa, and then converted into units of kgF/cm^2. This is because kgF/cm^2 is more commonly used in the industry and technical publications.

Figure 24 The water bath fitted with a Grant Optima thermostat/circulator.

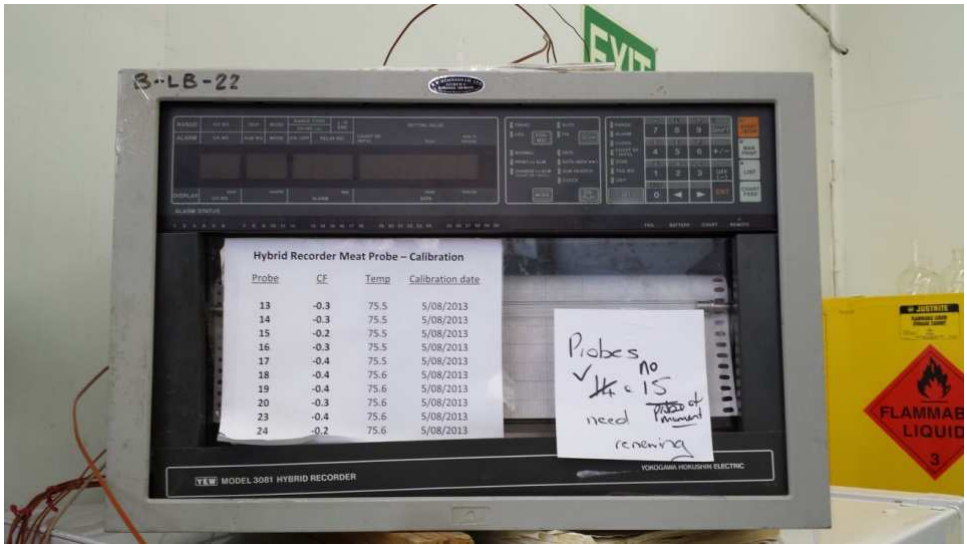

Figure 25 The hybrid recorder

Figure 26 MIRINZ tenderometer.

Data handling and statistical analysis

Statistical software and its application

Much preliminary data handling and inspection was performed with routines in Microsoft Excel, but for more insightful analysis, the R statistical software (Free Software Foundation) was used by Dr Martin Bader of Scion Limited, Rotorua. The use of R or any other sophisticated statistical software on this data set was beyond my capability and I freely acknowledge that. However, I made every attempt to understand what Dr Bader did and why he did it. R was applied to all analyses described below titled with 'Effects of...'. The statisticians report was very thorough and what is described in these sections is an abstracted version. A fuller description from Dr Bader of the logic behind the choice of analysis is shown in the Appendix.

Data preparation for pH and temperature decline analysis of two indicator muscles

The pH and temperature values of the two indicator muscles from the 45 carcasses were not taken at the same time for each animal. For example, if data from the first animal were taken at time T1, and the equivalent data from the 12th animal was taken about 25 minutes later. The following method was used to convert the raw data into a summary form.

First, the hours and minutes from slaughter were converted to decimal hours from slaughter and for each animal the values were plotted as continuous data up to 8 hours, e.g. pH (Y axis) against time in hours from slaughter (X axis) (Table **5**). After inspecting the data and trial curve fitting in Excel, it was shown that quadratic equations fitted the data best. These equations had the form:

$$\mathrm{Y} = \mathrm{Ax}^2 + \mathrm{Bx} + \mathrm{Constant}$$

where *Y* = the predicted temperature or pH, x = time from slaughter

For each stimulation treatment, there were 12 animals and each generating an equation for pH and temperature decline. To standardise the pH and temperature at each time interval, x = 1, 2, 3, 4, 5 and 6 hours were input into each quadratic equation, generating six values for each of *A*, *B* and *Constant* from the 12 carcasses (Table **5**). The means and standard deviations were calculated so that a single plot could be generated, with standard deviations as error bars for each of the two

indicator muscles, for pH and temperature, under the three treatments (Table **5**). Figure 27 shows a typical result of this data summary, in this case for Stimul 2 pH decline on *longissimus dorsi* muscle.

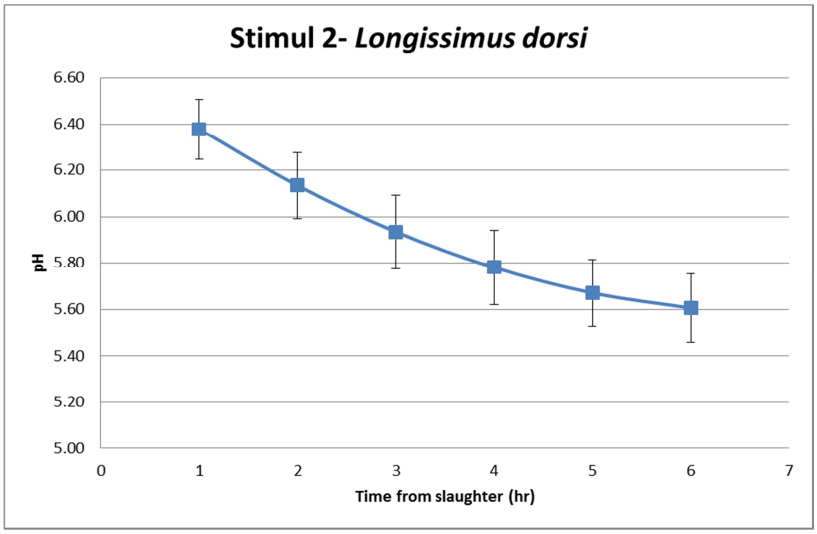

Figure 27 Typical pH decline curve with standard deviations, in this case for striploin in Stimul 2 from 12 carcasses.

Table 5 Data handling for pH and temperature decline in the two indicator muscles.

Sequence	Action	pH decline	Temperature decline
1	Input pH and temperature values for 15 animals as XY scatter. Categorise the animal by its weight: light, medium & heavy	Create 2 muscles x 3 treatments x 15 carcasses = 90 plots	Create 2 muscles x 3 treatments x 15 carcasses = 90 plots
2	Select 12 animals out of 15.	Create 2 muscles x 3 treatments x 12 carcasses = 72 plots	Create 2 muscles x 3 treatments x 12 carcasses = 72 plots
3	Convert time post slaughter into decimal hours and plot as X Y scatter plot	Create 2 muscles x 3 treatments x 12 carcasses = 72 plots	Create 2 muscles x 3 treatments x 12 carcasses = 72 plots
4	Fit quadratic equation	Get 12 values of *A*, *B* and *Constant* x 2 muscles x 3 treatments	Get 12 values of *A*, *B* and *Constant* x 2 muscles x 3 treatments
5	Calculate means and SD for *A*, *B* and *Constant*	Means and SDs result in plots for 2 muscles x 3 treatments = 6 plots	Means and SDs result in plots for 2 muscles x 3 treatments = 6 plots
6	Perform statistical analysis on polynomial coefficients	Analyse for 2 muscle and 3 stimulation treatment and their interactions	Analyse for 2 muscle and 3 stimulation treatment and their interactions
7	Plot calculated values of pH decline and temperature decline for each stimulation treatment and each indicator muscle; inspect plots for potential cold- or heat shortening		
8	Inspect curves in sequence 3 above to show which stimulation treatment results in the lowest variability in pH		

Effects of stimulation treatment and indicator muscle on pH and temperature decline in the hours following slaughter

The A, B and Constant values from the polynomial equation at each treatment were fitted into a linear mixed effect model to test the main effects and interactions between the stimulation methods and muscle, and the rate of pH and temperature decline. When there is a significant difference, a multiple comparison procedure, using Tukey contrasts, was used to identify where the significance lay.

Effects of carcass weight on pH and temperature decline in two indicator muscles

In each stimulation treatment, different coloured dots were used to indicate light, medium and heavy weight carcasses. The graphs were inspected if there is a relationship between carcasses weight and pH-temperature decline rate.

Effects of pH and temperature decline in two indicator muscles on meat quality data in the six muscles

The pH and temperature data were plotted against meat quality data and inspected for trends.

Data preparation for the effects of stimulation treatment on meat quality

Figure 28 shows the typical spreadsheet format for these data. Treatment 1 was Stimul 1, the muscle was BLD (*infraspinatus*) from two carcasses, assigned for 2 x 4 ageing days. The data in purple are the meat quality data from that muscle. (The narrowed data in blue are for an indicator muscle.)

Treatment	Body number	Dentition	Weight	Muscle	p:ph:eT	Position	Age days	Driploss%	L*	a*	b*	Hue angle	Saturation	Delta E	Japan	Tenderness	TenderSTD
1	1	0	247.5	BLD		1	1	0.000	36.38	17.68	15.69	0.73	23.64	43.39	4.13	6.44	3.4
1	1	0	247.5	BLD		2	5	0.629	30.4	25.98	23.81	0.74	35.24	46.54	5.28	5.89	3.1
1	1	0	247.5	BLD		3	21	1.215	32.69	22.81	21.86	0.76	31.59	45.46	4.83	4.44	2.4
1	1	0	247.5	BLD		4	3	0.462	32.59	21.65	19.22	0.73	28.95	43.59	4.89	6.86	3.6
1	2	4	273.5	BLD		1	14	0.806	28.99	24.25	22.36	0.74	32.99	43.91	5.62	4.09	2.2
1	2	4	273.5	BLD		2	7	0.420	32.29	23.01	22.25	0.77	32.01	45.47	4.91	5.14	2.7
1	2	4	273.5	BLD		3	5	0.430	30.64	24.84	23.64	0.76	34.29	45.99	5.24	4.69	2.5
1	2	4	273.5	BLD		4	14	1.203	30.2	23.58	21.78	0.75	32.10	44.07	5.37	3.74	2.0

Figure 28 Typical structure of the spreadsheet serving as the master document for analysis. Columns in purple contain the meat quality data for the BLD muscle from two carcasses (Body number) and one stimulation treatment (Stimul 1). The narrowed data in blue are for an indicator muscle.

Effects of stimulation treatment, muscle and days after slaughter on drip loss

Inspection of the drip loss curves (Figure 48) indicated that an asymptotic equation of the form fitted the data well, realising that at Day 0, the percent drip loss was zero:

$$y = Asym * (1 - \exp(-\exp(lrc) * x))$$

In this equation y is percent drip, Asym is the theoretical asymptotic value of percent drip, lrc is the logarithm of the rate constant, and x is the days after slaughter (ageing) to 21 days. Carcass number was a random term.

Effects of stimulation treatment, muscle and days after slaughter on Hunter a* and b* values, and saturation

Inspection of the Hunter a* values (Figure 58) indicated that an asymptotic equation of the form fitted the data well:

$$y = Asym + (R_0 - Asym) * \exp(-\exp(lrc) * x)$$

In this equation, Day 0 (R_0 in this equation) was not zero. Here, y and R_0 are Hunter value a*, lrc is the logarithm of the rate constant and x is the days of ageing to 21 days – both just as for Hunter a*. Carcass number was a random term. Stimul 1 & 3 were pooled using this equation.

This equation has another non-linear form of a quadratic equation, which was used for Stimul 2, where a humped relationship (Figure 60 in Appendix) was found for Hunter a* values with time:

$$y = \alpha (x - \beta)^2 + \gamma$$

where α = indicates shape of the hump, β = x-value when the maximum a* value occurs, and γ is the maximum a* value (Figure 60 in Appendix). The higher the α value, the more humped is the curve. Hunter b* values and the saturation were similarly humped over days of ageing, so the same equation was applied to these data too.

Effects of stimulation treatment, muscle and days after slaughter on Hue angle

Hue angle is defined as $\tan^{-1}(b^*/a^*)$, and is the colour that is perceived by the eye. Because the functions of a* and b* were usually humped positively, so it was likely that plots of Hue angle against days after slaughter would approximate straight lines. Figure 50 proved to be the case.

Effects of stimulation treatment, muscle and days after slaughter on Hunter L and Japan colour assessment*

Hunter L* measures the percent total light reflectance and 'Japan colour score' is a subjective assessment of colour using meat-coloured tiles as the reference. The relationship between L*, and Japan colour score, and time after slaughter showed some curvatures. Therefore, a flexible generalised additive mixed model (GAMM) was used to test for differences between stimulation methods. For both L* and Japan colour score, it was found that the curvature was solely due to Stimul 2; while Stimul 1 and 3 showed similar linear patterns.

Consequently, the pooled responses of Stimul 1 and 3 were modelled using a linear mixed-effects model fitted by restricted maximum likelihood. The model contained stimulation, muscle, days after slaughter (days of aging) and their interactions as fixed terms and carcass number as a random term. The more curved responses of Stimul 2 was modeled using a GAMM, which includes muscle as factor, a smoother for days after slaughter and carcass number as a random term.

Effects of stimulation treatment, muscle and days after slaughter on cooked meat shear force

Inspection of plots showed a usually negative response of shear force, but with some curvature (Figure 55). Therefore, as for L* and Japan above, a GAMM was used to accommodate the curvature seen in the development of shear force over time. The model contained stimulation and muscle as factors and a smoothing term for time (days after slaughter). Carcass number was a random term.

To test whether the development of reduced shear force over time followed a common pattern across the three stimulation methods, a model with one common smoother was compared to a model allowing the smoother term to vary with stimulation. It turned out that pattern of the relationship between shear force and time after slaughter

was similar across stimulation method, thus favouring the model with one common time smoother across stimulation methods. However, the development of reduced shear force over time varied significantly across muscles; therefore the model with individual time smoothers for each muscle is more favourable.

Layout of results and discussion

The results and discussions logically fit into two chapters. **Chapter 4** reports the experiments with the indicator muscles. There are three parts to this Chapter, namely items 6, 7 and 8 in Table **5**:

6) Plot calculated temperature decline values against pH decline for each stimulation treatment and each indicator muscle (Part 1); inspect plots for potential cold- or heat shortening on light, medium and heavy weight carcasses.
7) Effects of stimulation treatment and indicator muscle on pH and temperature decline rates in the hours following slaughter (Part 2).
8) Inspect pH and temperature curves in 6) to indicate which stimulation treatment results in the lowest variability in pH (Part 3)

Chapter 5 will focus on the effects of stimulation (3 treatments) and muscle (6 of these in Table 4) on meat quality attributes as a function of days after slaughter.

Chapter 4 Results and discussion

Part 1 pH-temperature profiles and the risk of heat shortening

As detailed in Table 2 in Material and Methods section, there were three stimulation treatments:

Stimul 1 was a continous 15 Hz immobilisation for 40 seconds

Stimul 2 was 4 seconds of 15 Hz, followed by 36 seconds of 400 Hz

Stimul 3 comprised Stimul 2 plus Smart Stimulation at 30 minutes post slaughter

The pH and temperature readings were taken hourly on the anterior end of *longissimus dorsi* and deep *semimembranosus* muscles on the carcass during refrigeration. For each treatment, I colour coded the carcass dressed weights into light, medium and heavy and plotted pH-temperature graphs for the two indicator muscles. From these graphs, it was possible to show which stimulation treatment – if any – caused heat or cold shortening, and if the carcass dressed weight had an effect on the pH-temperature profile with respect to heat or cold shortening.

According to MSA grading requirements (Figure 10), to avoid heat shortening, the meat pH should not be lower than pH 6 when the meat temperature is above 35℃; whereas to avoid cold shortening, the meat pH should be lower than 6 when the meat temperature is below 15℃. On the figures, the heat shortening window is indicated in red square; whereas the cold shortening window is in blue square.

The pH-temperature profile in Figure 29 for Stimul1 is clearly different from those of Figure 31 and Figure 32, where there are datapoints above 35℃ for Figure 31 and Figure 32, but not for Figure 29. This was because of an unforseen and unfortunate health and safety concern during the data collection for Stimul 1, where the use of ladder in the chiller was not permitted. As a result, the first measurements did not start until the meat had dropped below 35℃. Inspe ction of Figure 29 suggests that if the data were available, there would be a risk of heat shortening. To check for possible heat shortening by Stimul1, historical data of Silver Ferns Farms and Carne Technologies were examined (Figure 30 & Table **6**) .

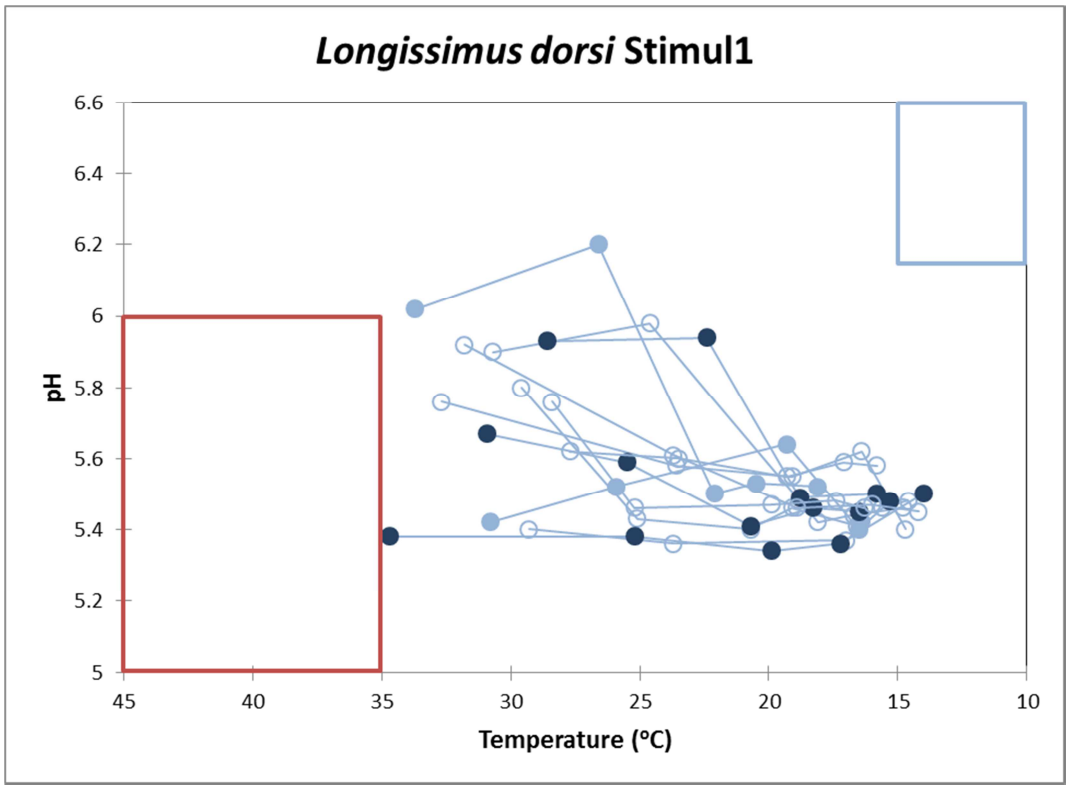

Figure 29 pH-temperature profile for *longissimus dorsi* with Stimul1. Blank dots = light weight carcasses; light blue = medium weight; dark blue = heavy weight. In this figure and similar plots, the red rectangle represents the likely region for heat shortening and the blue rectangle for cold shortening.

According to Carne, with continous 15 Hz (Stimul1), although the pH values were variable, it was found that the mean pH decline was very rapid as a result of the immobilisation (Table **6**). Indeed, a proportion of the carcasses were in or very near to ultimate pH at 50 minutes. Additionally, they observed some pale *Semimembranosus* muscles in the boning room, which was evidence of heat shortening. Historical work by Silver Fern Farms personnel also demonstrated low pH (less than pH 6) at chiller entry (Figure 30).

Table 6 *Longissimus dorsi* pH at chiller entry. Data from by Carne Technologies using Stimul1	
Mean pH	5.7
Maximum/minimum pH	6.4/5.4

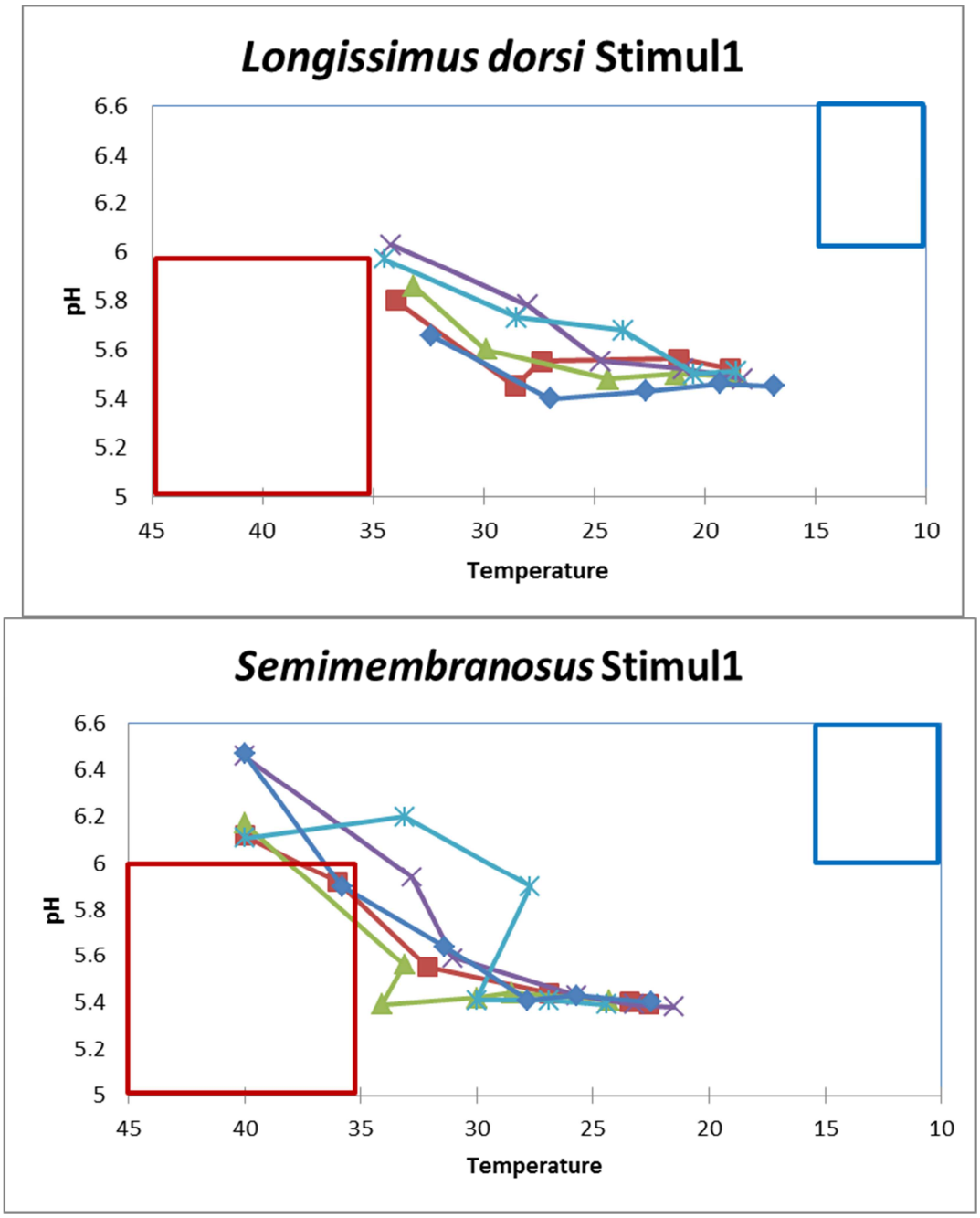

Figure 30 Historical data by Silver Fern Farms on the pH-temperature profile with Stimul1, for *longissimus dorsi* and *semimembranosus*. Data are for five carcasses.

The conclusion from data in Figure 29, Figure 30 and Table **6** is clear. There is a high risk of heat shortening from Stimul1.

With *longissimus dorsi* as the indicator muscle, the pH-temperature profiles from Stimul 2 and Stimul 3 showed no risk of heat shortening. For all stimulation treatments (Figure 29, Figure 31 and Figure 32), the carcass dressed weight

distribution was widespread and did not appear to have signficant affect on the pH-temperature profile for *longissimus*.

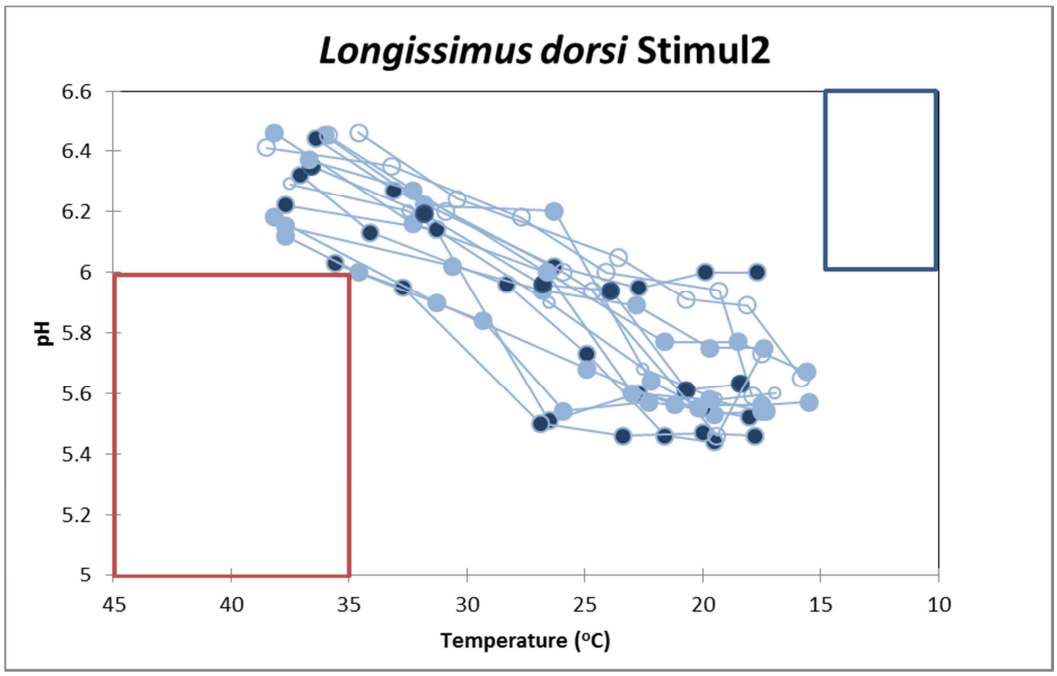

Figure 31 pH-temperature profile for *longissimus dorsi* with Stimul2. Blank dots = light weight carcasses; light blue = medium weight; dark blue = heavy weight.

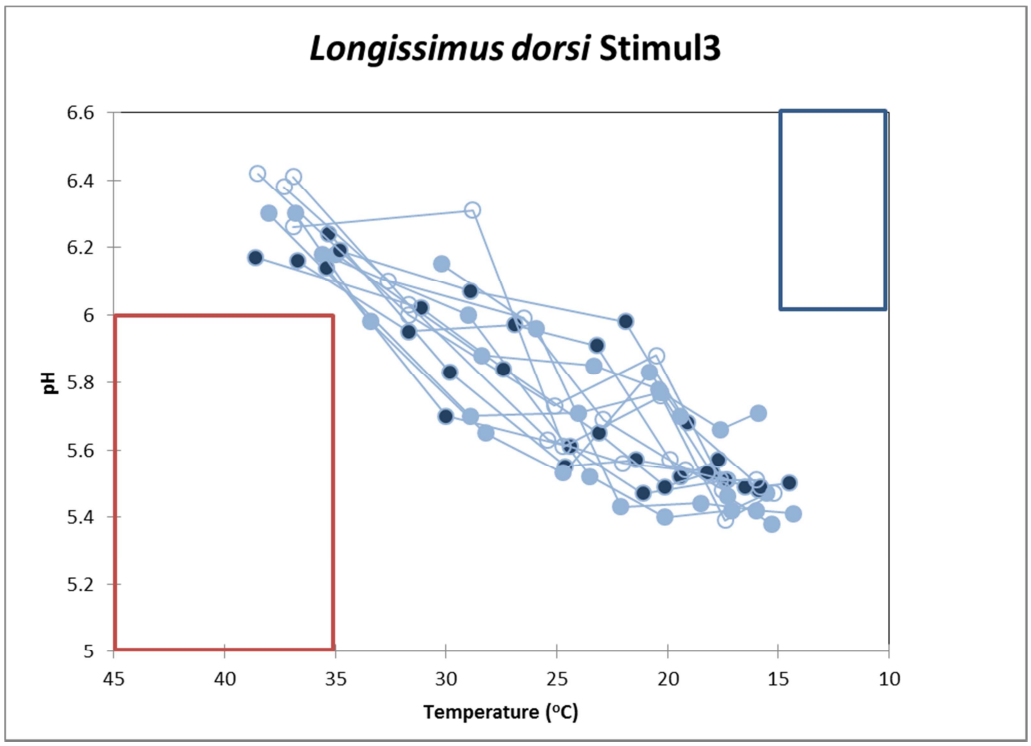

Figure 32 pH-temperature profile for *longissimus dorsi* with Stimul3. Blank dots = light weight carcasses; light blue = medium weight; dark blue = heavy weight.

With *semimembranosus* as the indicator muscle, the limited Stimul 1 data again suggested a potential risk of heat shortening (Figure 30; Figure 33). For Stimul 2 and Stimul 3, only a few carcasses showed a risk of heat shortening. For all stimulation treatments (Figure 33, Figure 34 and Figure 35), the carcass dressed weight distribution was widespread and did not appear to have signficant affect on the pH-temperature profile for *semimembranosus*.

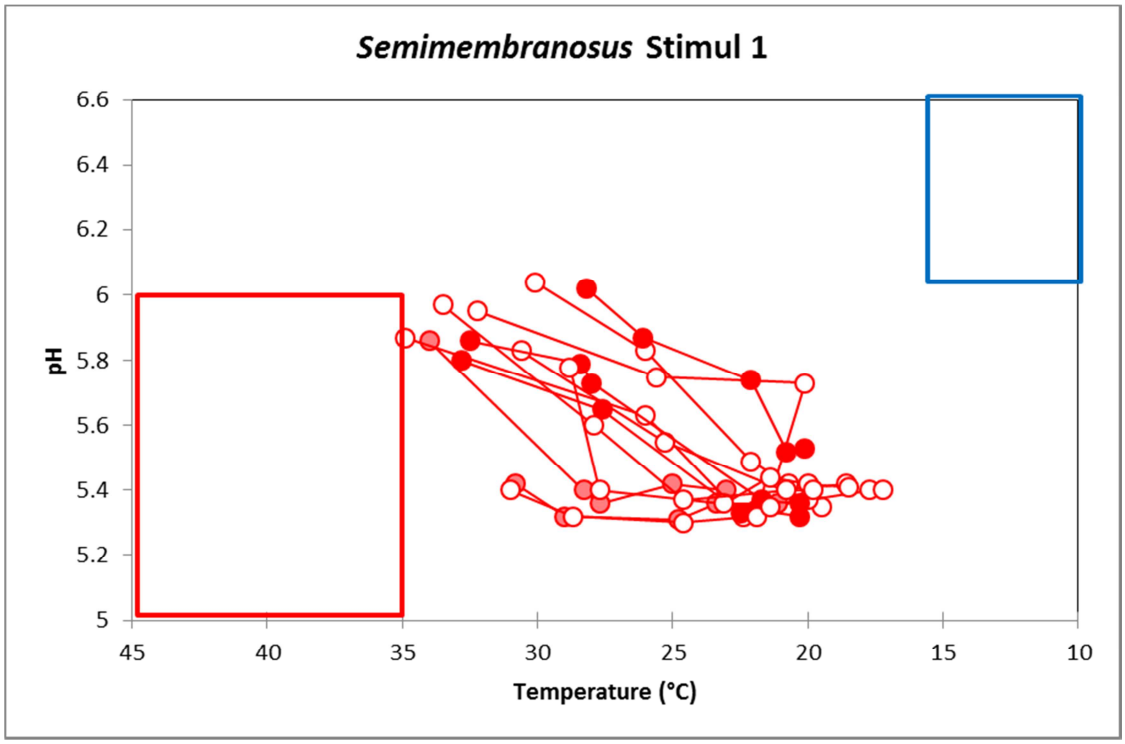

Figure 33 pH-temperature profile for *semimembranosus* with Stimul1. Blank dots = light weight carcasses; light red = medium weight; dark red = heavy weight.

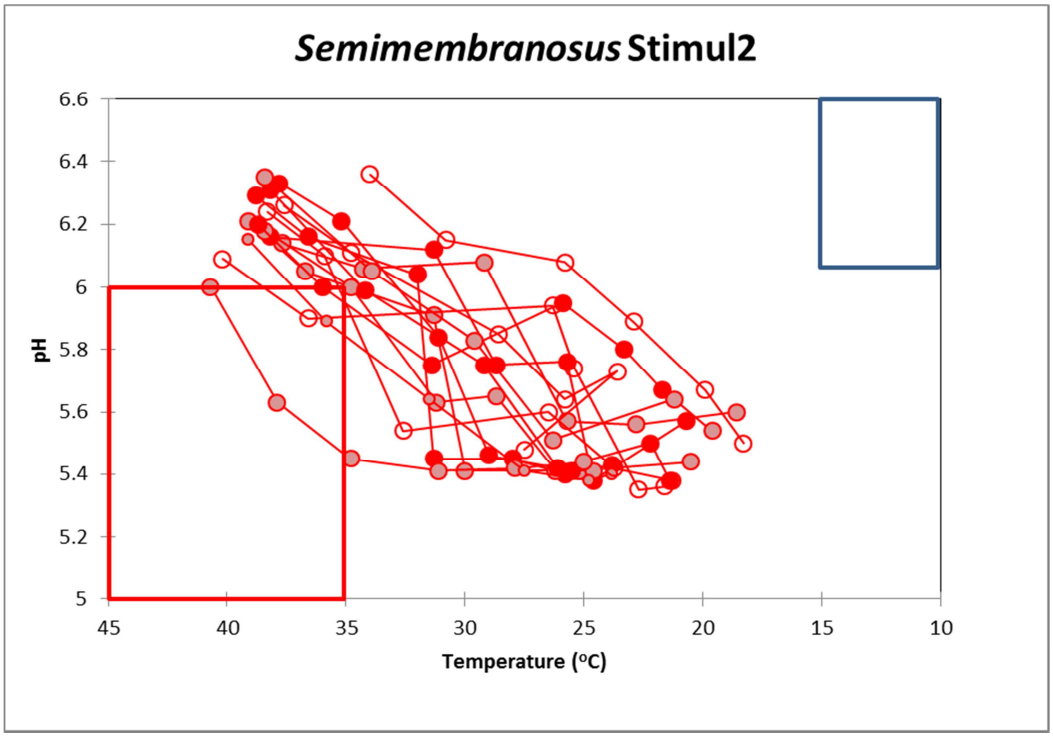

Figure 34 pH-temperature profile for *semimembranosus* with Stimul2. Blank dots = light weight carcasses; light red = medium weight; dark red = heavy weight

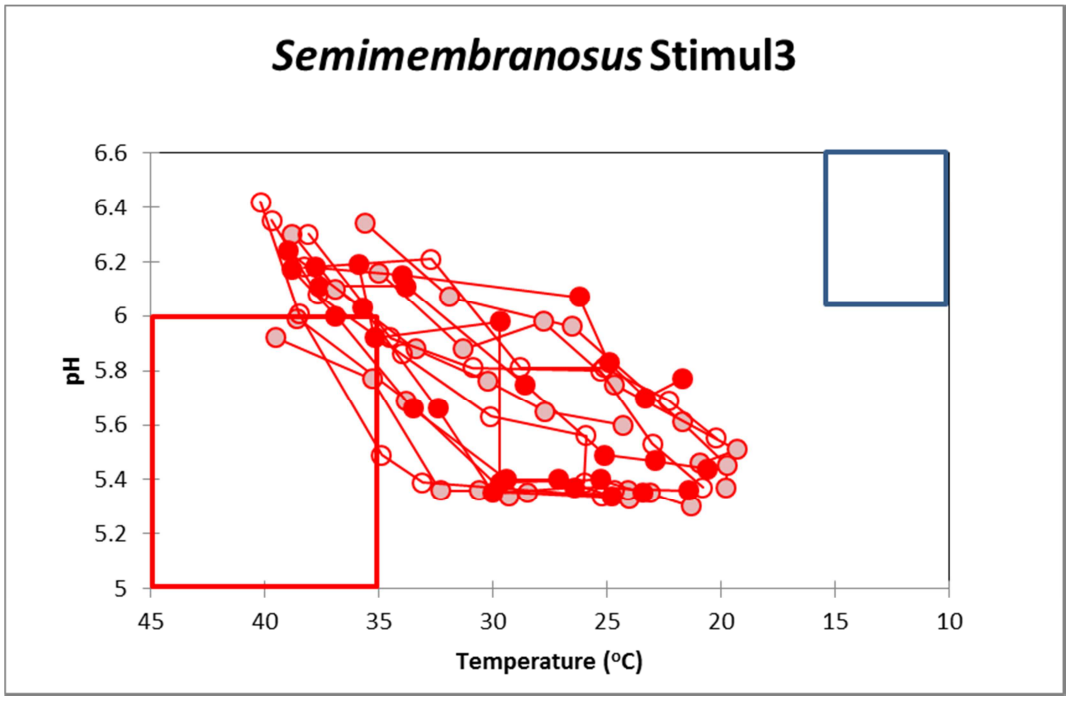

Figure 35 pH-temperature profile for *semimembranosus* with Stimul3. Blank dots = light weight carcasses; light red = medium weight; dark red = heavy weight.

The six scatter graphs discussed above could also be condensed into graphs plotting the pH-temperature profile means. However, the way the data were recorded differed in the time of sampling. This means that the points in the six scatter plots were actual pH-temperature values at any time they might be measured after slaughter. In contrast, the means shown in Figure 36 and Figure 37 were derived from the quadratic curve fitting methods described in Chapter 3.

Figure 36 and Figure 37 show the average pH-temperature of the two indicator muscles under different stimulation treatments. In Figure 36, when the meat temperature was higher than 25℃, the pH on Stimul 1 was the lowest, followed by Stimul 3, which is about 0.1 pH unit lower than Stimul 2. As temperature fell below 25℃, the differences between the treatments narrowed.

In Figure 37, the pH-temperature profiles for *semimembranosus* in all stimulation treatments were similar. Although not immediately obvious by inspection, at any given temperature, the pH values for *semimembranosus* were lower than those for *longissimus* (Figure 37). This is because the percentage of Type I fibers is higher in *longissimus dorsi* than in *semimembranosus* muscles (Hunt & Hedrick, 1977). Muscles with higher Type I fibers respond less intensively to electrical stimulation.Since the pH-temperature profile of longissimus dorsi in Stimul 1 was significantly within the heat shortening window, the more rapid pH decline in *semimembranosus* indicates that this muscle would also pose a risk of heat shortening.

Finally, a comparison of the three *longissimus* graphs with the three *semimembranosus* graphs (Figure 36 & Figure 37) suggests that *semimembranosus* was slightly at more risk of heat shortening for all stimulation treatments.

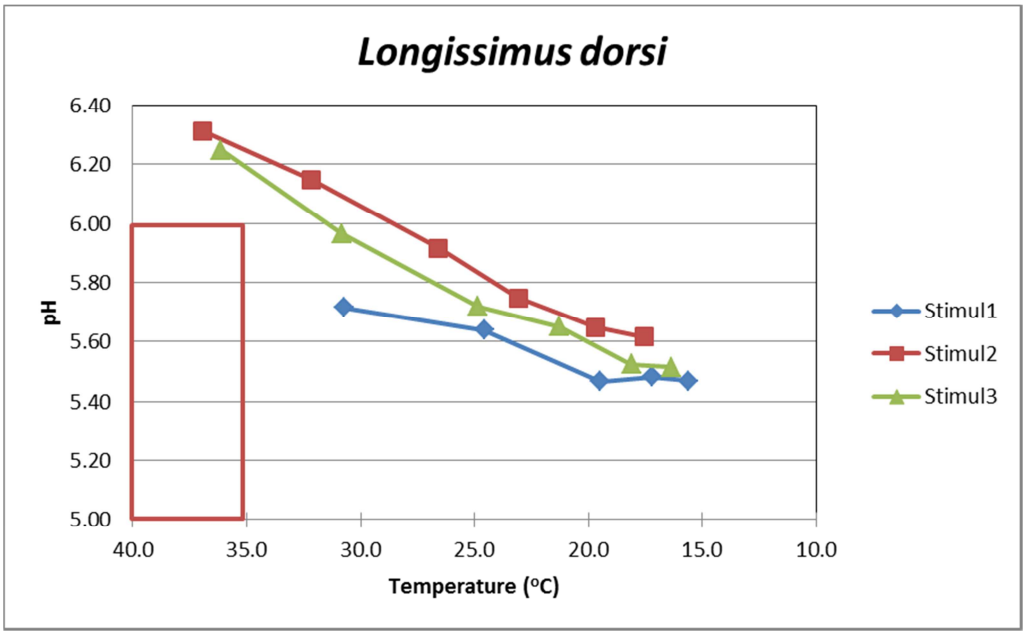

Figure 36 Mean pH-temperature profile for *longissimus* under three stimulation conditions.

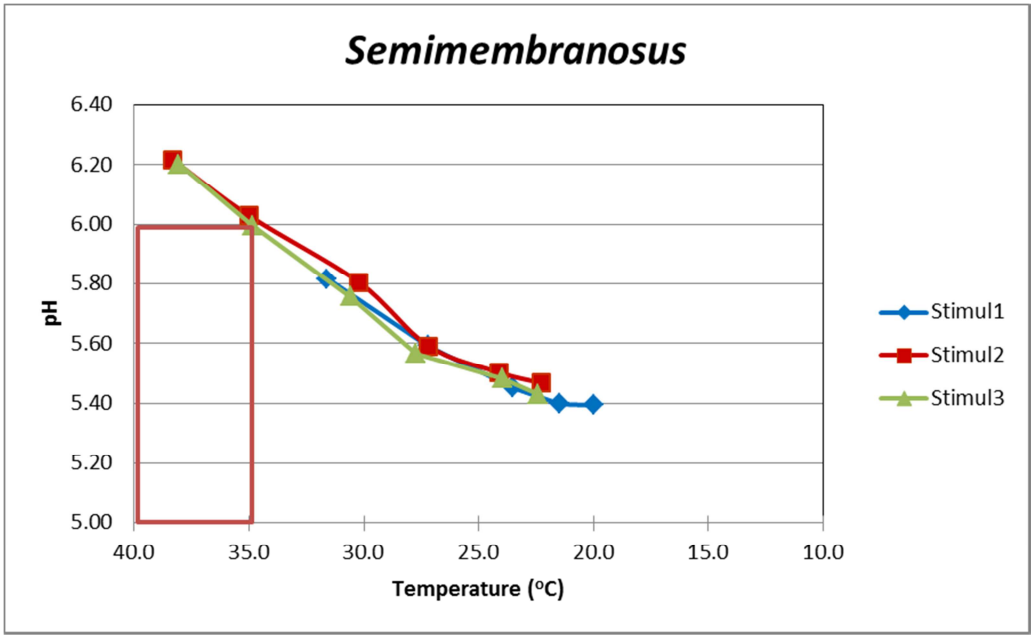

Figure 37 Mean pH-temperature profiles for *semimembranosus* under three stimulation conditions.

Part 2 pH and temperature decline rates and their variability in the hours following slaughter

Using quadratic equations fitted to raw data (Chapter 3), it was a simple matter to plot graphs of pH and temperature versus time. With the equation for each carcass, the predicted values of pH and temperture could be calculated at set times after slaughter, hours in this case. Means and standard deviations for 12 carcasses were calculated at these times. After plotting means as a scatter plot in Excel, the points were joined by straight lines to create Figure 38 and Figure 39; standard deviations are not shown for clarity.

For both indicator muscles in the first 5 hours after slaughter, Stimul 1 showed lower pH values than the other two treatments, but the differences were negligible by 6 hours. In Figure 38, the Stimul 3 showed a slightly lower pH than Stimul 2. However, this pattern was reversed for *semimembranosus* (Figure 39) Was this reversal significantly different? Table 7 showed that it was not.

In Table 7, the three terms of the quadratic equations – the squared term (RatepHtxx), the linear term (RatepHtx) and the constant (RatepHtcon) – have been statistically tested for the effect of stimulation. Of the three terms, linear term RatepHtx is the most important because it dominates the rate of pH fall. Stimulation had an effect on RatepHtx (P = 0.045), but the muscle effect was not significant (P = 0.570), and there was no interaction (P = 0.456).

Figure 40 shows the values of RatepHtx for the two muscles and 3 stimulation treatments. A multiple range comparison showed that the only significant difference (P = 0.031) was between Stimul 1 and Stimul 3 (Figure 40). (The Tukey contrasts for this are in Table 25, Appendix.)

Although Stimul 3 showed the steepest decline, what this analysis does not show is the likely rapid pH fall for Stimul 1 in 2 hours after slaughter. This is because the data were unavailable in that period because of health and safety issues. If that data were available it is possible that the term RatepHtxx (squared term) would have become significant for stimulation treatment.

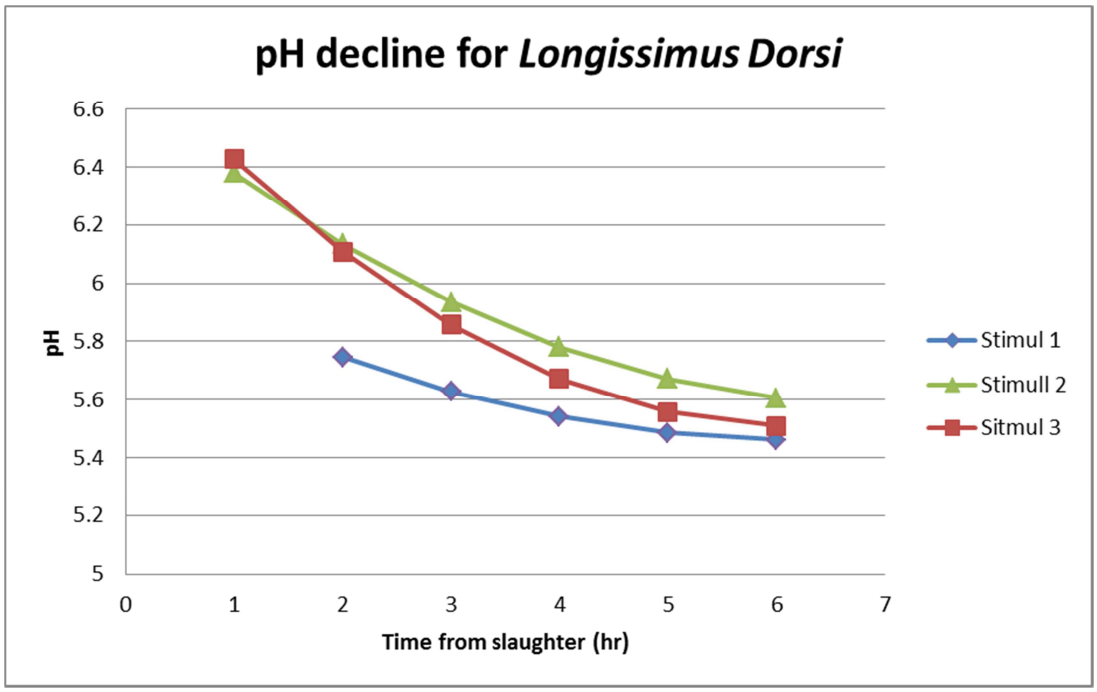

Figure 38 pH decline curve for *longissimus* (n = 12 for each stimulation treatment).

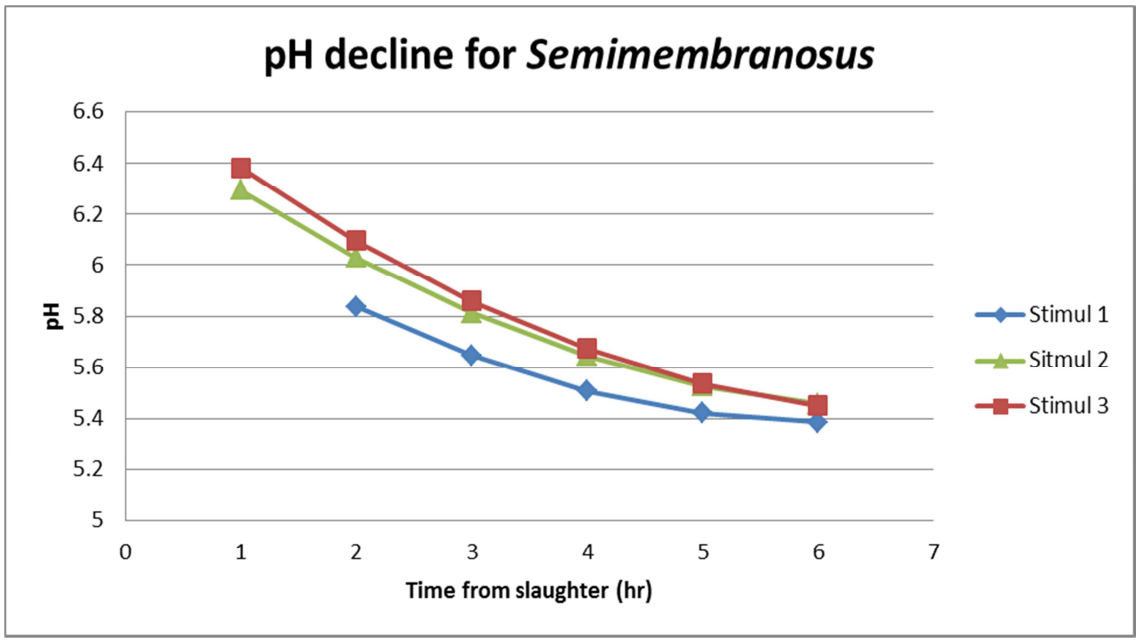

Figure 39 pH decline curve for *semimembranosus* (n = 12 for each stimulation treatment)

Table 7 Results from linear mixed effects models with a carcass random term testing the effects of stimulation treatment, indicator muscle group and their interaction on the rate of pH decline.

Parameter	Degree freedom (number)	Degree freedom (den)	F	P	Significance
RatepHtxx					
Intercept	1	55	54.58	< 0.001	***
Stimulation	2	55	1.526	0.227	
Muscle	1	55	0.089	0.767	
Stimul. x muscle	2	55	0.655	0.523	
RatepHtx					
Intercept	1	55	113.19	< 0.001	***
Stimulation	2	55	3.293	0.045	*
Muscle	1	55	0.327	0.570	
Stimul. x muscle	2	55	0.797	0.456	
RatepHtcon					
Intercept	1	55	26470	< 0.001	***
Stimulation	2	55	12.78	< 0.001	***
Muscle	1	55	1.02	0.317	
Stimul. x muscle	2	55	1.29	0.283	

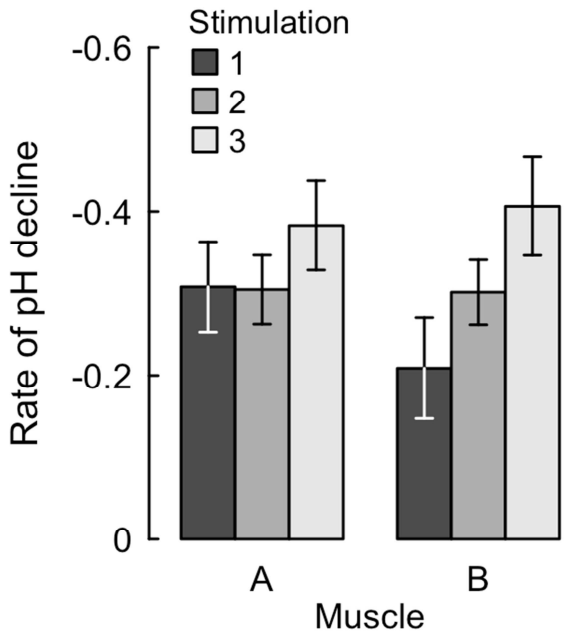

Figure 40 The rate of pH decline (RatepHtx*)* for the *longissimus* (A) and *semimembranosus* (B). The only significant difference ($P < 0.05$) was between Stimul 1 and Stimul 3, and that can be inferred by inspection of Figure 38 and Figure 39.

Next, I will examine the parallel data for the rate of temperature decline. The linear mixed effects models showed that there was a significant muscle effect ($P < 0.001$) on the RateTtxx and the more important RateTtx, but no significant effect of stimulation treatment (Table 8). The *semimembranosus* (Muscle B) cooled more rapidly than the *longissimus* (Muscle A). This is very clear from Figure 41. As the carcasses were hung by the Achilles tendons in the chillers, the s*emimembranosus* muscles were more exposed to the cooling fans that were blown down from the ceiling.

Table 8 Results from linear mixed effects models with a carcass random term testing the effects of electrical stimulation (ES) method, muscle group and their interaction on the rate of temperature decline.

Parameter	Degree freedom (number)	Degree freedom (den)	F	P	Significance
RateTtxx					
Intercept	1	55	466.493	< 0.001	***
Stimulation	2	55	2.216	0.119	
Muscle	1	55	20.457	< 0.001	***
Stim. x muscle	2	55	1.390	0.258	
RateTtx					
Intercept	1	55	1258.391	< 0.001	***
Stimulation	2	55	1.564	0.218	
Muscle	1	55	33.176	< 0.001	***
Stim. x muscle	2	55	1.454	0.243	
RateTtcon					
Intercept	1	55	14454.685	< 0.001	***
Stimulation	2	55	5.536	0.007	**
Muscle	1	55	3.857	0.055	.
Stim. x muscle	2	55	2.075	0.135	

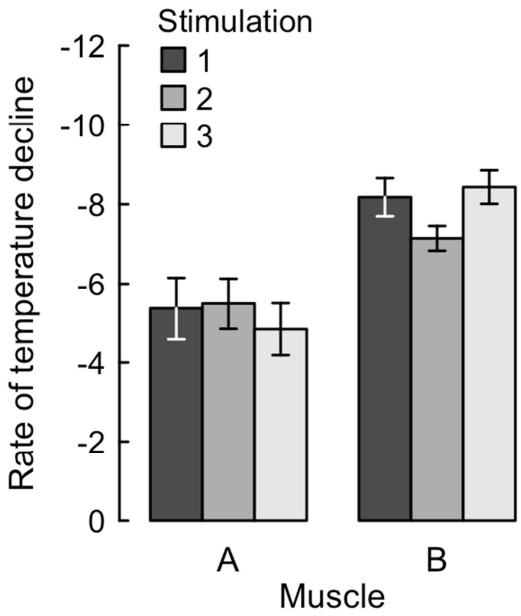

Figure 41 The rate of temperature decline (RateTtx*)* for the *longissimus* (A) and *semimembranosus* (B). The only significant difference (P < 0.001) was between the muscles. Stimulation (Stimul 1, 2 and 3) had no effect.

Discussion

From an industry prospective, the most effective electrical stimulation system would be one that – on average – not only avoided heat and shortening but also had the lowest variability in pH. Quite simply, the meat quality would be more consistent. To check for this, the individual curves in Figure 38 and Figure 39 have been redrawn with standard deviations to indicate the variability.

For *longissimus* (Figure 42, Figure 43 and Figure 44), it is obvious that the variability in the early hours after slaughter is greatest in Stimul 1 and least for Stimul 3. The latter result suggests that the Smart Stimulation system has been effective in regulating pH fall in that muscle.

For *semimembranous* (Figure 45, Figure 46 and Figure 47), the theme of greater variability for Stimul 1 was again clear. With Stimul 2, the variability was roughly the same as observed for *longissimus*; but for Stimul 3 it was markedly greater.

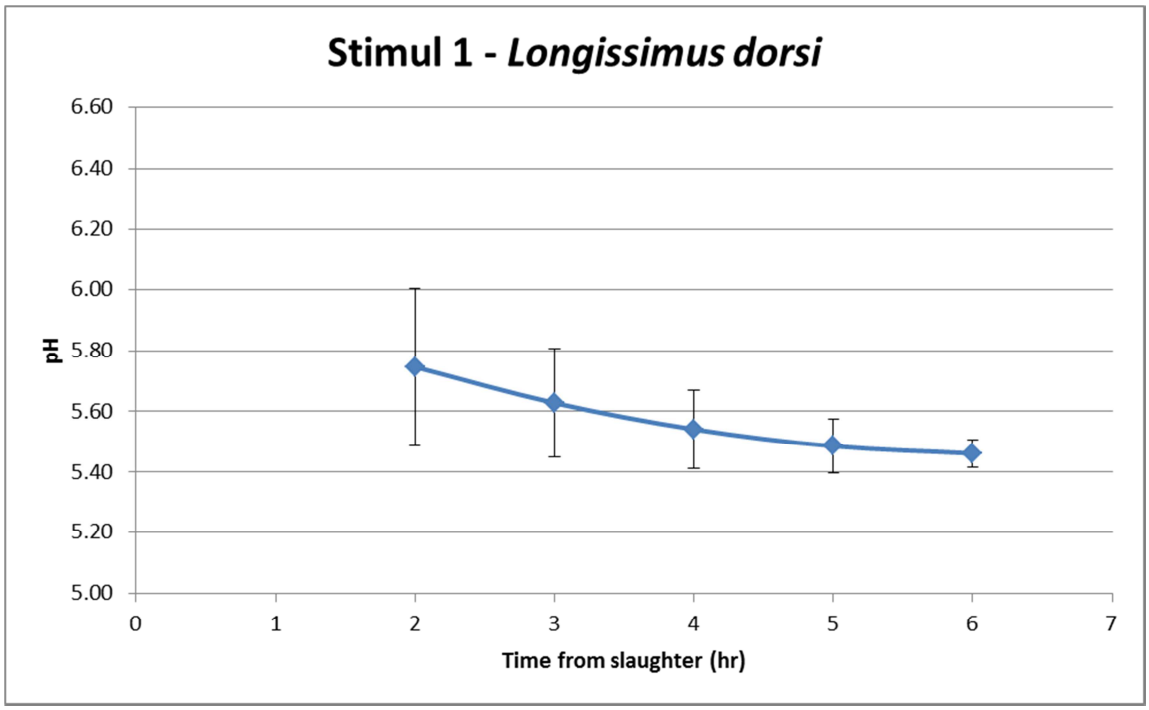

Figure 42 pH decline curve with standard deviations for Stimul 1.

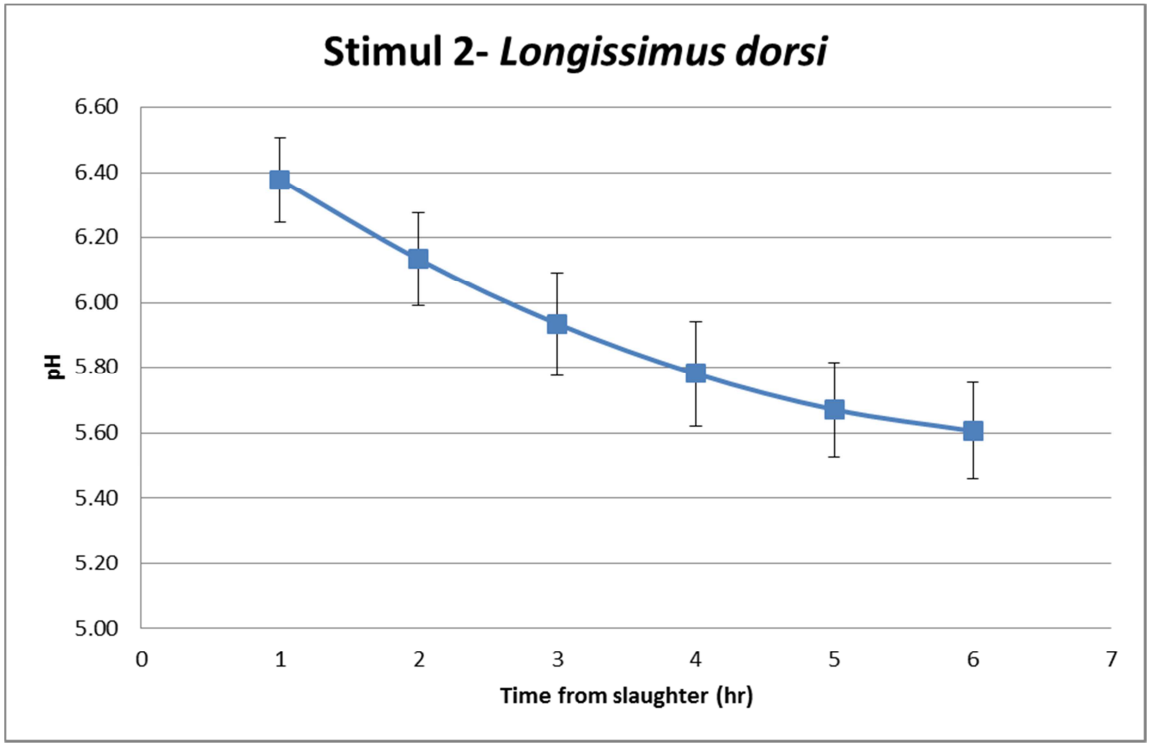

Figure 43 pH decline curve of *longissimus* with standard deviations for Stimul2.

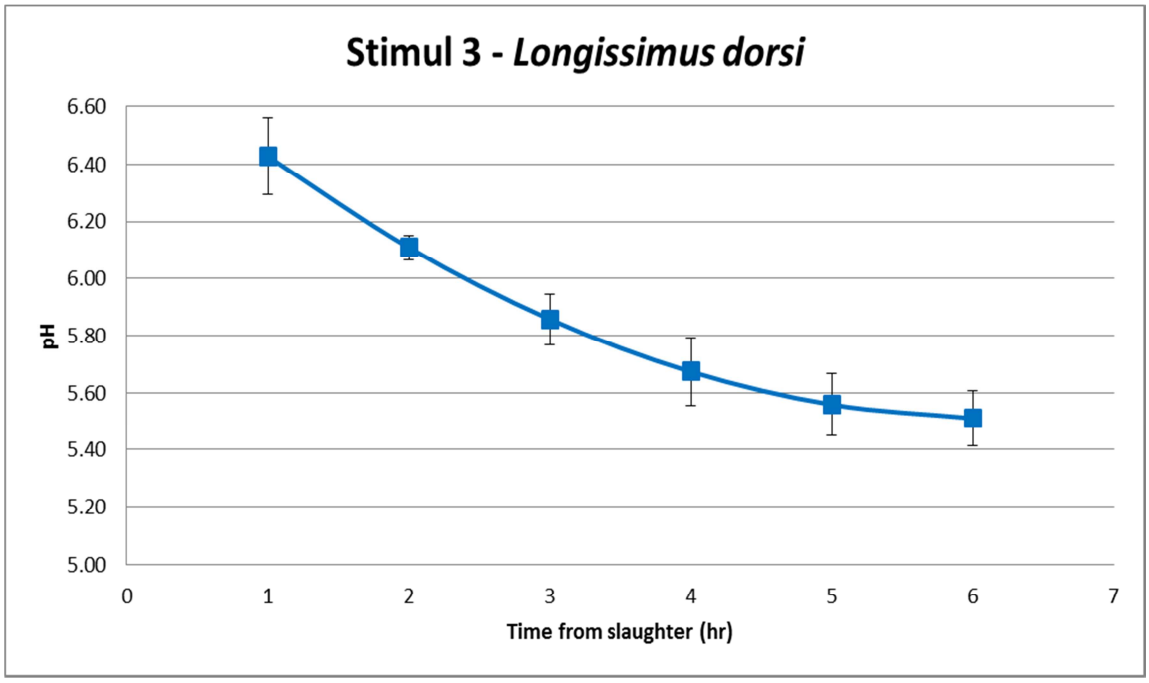

Figure 44 pH decline curve of *longissimus* with standard deviation for Stimul3.

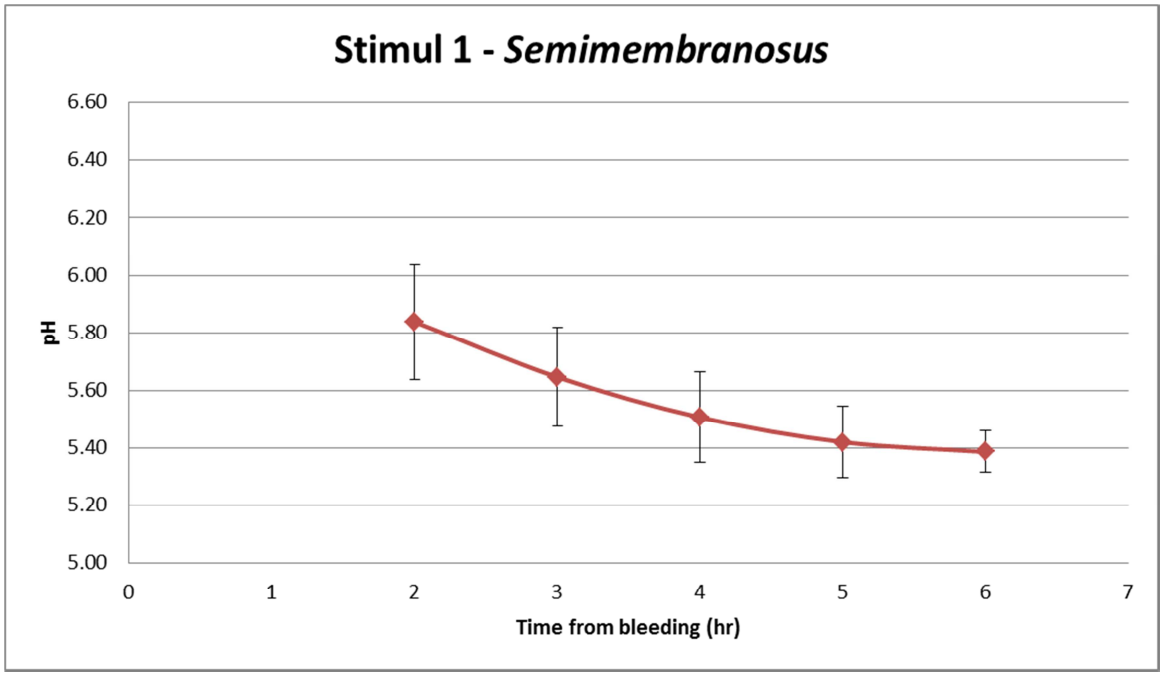

Figure 45 pH decline curve of *semimembranosus* with standard deviations for Stimul1.

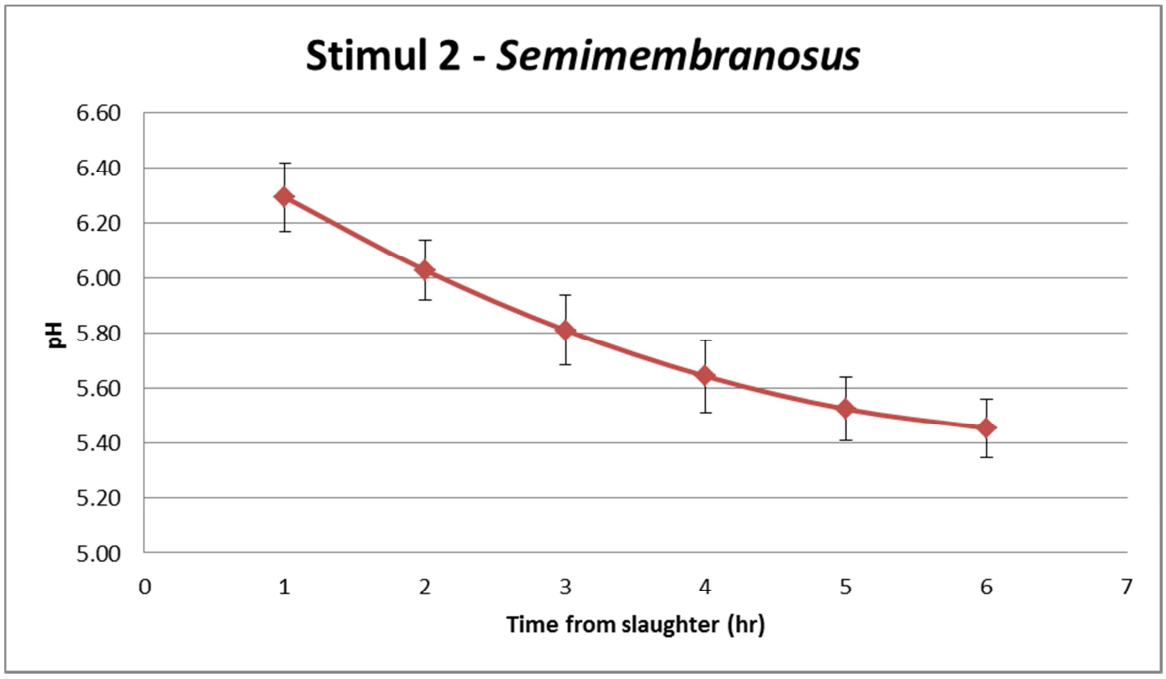

Figure 46 pH decline curve of *semimembranosus* with standard deviations for Stimul2.

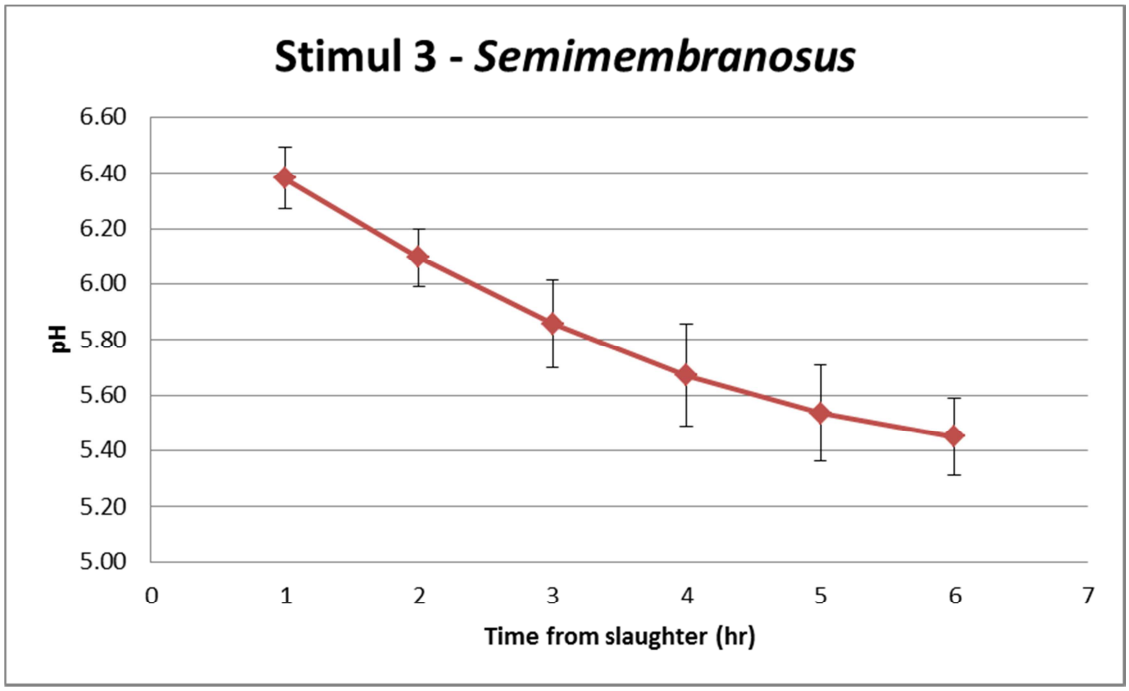

Figure 47 pH decline curve of *semimembranosus* with standard deviations for Stimul3.

Some conclusions can be drawn. Stimul 1 has the highest stimulation effect on both indicator muscles. The pHs of *longissimus* and *semimembranosus* muscles were already below pH 6.0 at 2 hours post slaughter, putting the meat in the risk of heat shortening. Stimul 2 and 3 are much better treatments, in which the meat is getting enough stimulation to avoid cold shortening window and at the same time not over-stimulated to the point of heat shortening. In respect of Stimul 2 and 3, the rate of pH decline was closely similar for *semimembranosus*, but greater pH decline from Stimul 3 in *longissimus*. Stimul 3 includes Smart Stimulation and the implication is that application of its stimulation bar to *longissimus,* would only accelerate the glycolysis in that and neighbouring muscles, but not in *semimembranosus*. This would explain the greater variability of pH in the (unaffected) *semimembranosus*.

Chapter 5 Results and discussion
Effects of stimulation treatments on meat quality

Effects of stimulation treatment, muscle and days after slaughter on drip loss

The commercially important parameter, drip loss increased asymptotically with time but there was no significant difference between electrical stimulation methods (P = 0.157). However, there was a significant difference between muscles (P < 0.001) (Figure 48 a – f).

On Figure 48 and Table 9, 21 days after slaughter, *gluteus medius* (RUMH) showed the greatest drip loss of around 7.5%, which was significantly greater than the drip loss observed in *rectus femoris* (KNUCK), *longissimus dorsi* (STRL) and *infraspinatus* (BLD). The BLD has the lowest drip loss of roughly 4% (Figure 48 and Table 9). *Gluteobiceps* (RUMC) and *psoas major* (TEND) also showed relatively high drip losses of around 6% but did not differ significantly from any other muscle (Figure 48c and f, Table 9).

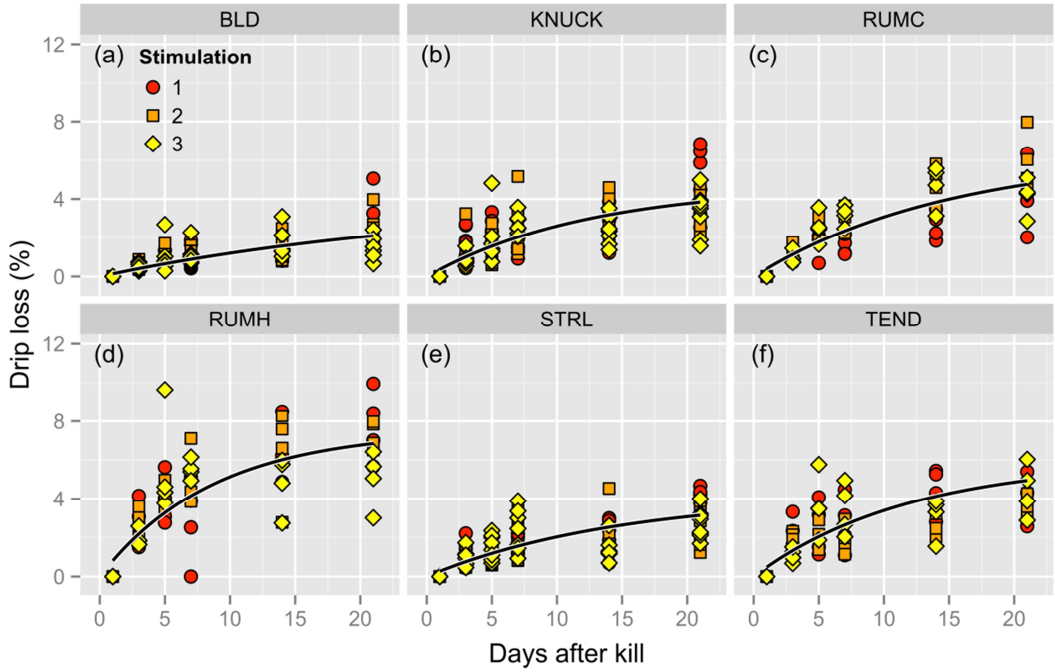

Figure 48 Drip loss percent as a function of days after slaughter by muscle (a – f) and electrical stimulation methods (red circles = Stimul1, orange squares = Stimul2, yellow diamonds = Stimul3). The lines represent fits from a nonlinear mixed-effects model with individual parameter estimates for each muscle based on an asymptotic function.

Table 9 Parameter estimates from a non-linear asymptotic meat drip loss model with separate parameter estimates for each muscle (values in brackets are standard errors of the parameter estimates). Model parameters: *Asym* = upper asymptote, the maximum and final predicted drip loss % at infinite time *lrc* = logarithm of the rate for drip loss to attain maximum value (determines the curvature). Different lower case letters indicate significant differences at P < 0.05.

Muscle	Model parameters (SE)	
	Asym	*lrc*
BLD	3.92 (1.11) [a]	-3.31 (0.37) [a]
KNUCK	4.70 (0.60) [a]	-2.52 (0.20) [a]
RUMC	6.32 (1.12) [ab]	-2.71 (0.27) [a]
RUMH	7.53 (0.78) [b]	-2.17 (0.19) [a]
STRL	4.16 (0.61) [a]	-2.70 (0.22) [a]
TEND	5.96 (0.88) [ab]	-2.48 (0.24) [a]

Effects of stimulation treatment, muscle and days after slaughter on Saturation

Saturation measures colour intensity and combines colour indicator a* (redness) and b* (yellowness) in the function $\sqrt{(a^{*2} + b^{*2})}$, which is the length of the hypotenuse linking the orthogonal a* and b* values. Saturation is more useful in practical terms than a* and b* as stand alone parameters. The larger the saturation value, the more intense the colour or hue. Since a* and b* were often best described by quadratic functions (Figure 58 and Figure 61 in Appendix), it was expected that a quadratic function would also best describe saturation as a function of days after slaughter. This proved to be the case (Figure 49).

The saturation development showed a curvilinear response over time, reaching peak values approximately 14 days after slaughter (Figure 49). The stimulation treatments had no effect on saturation development over time (P = 0.062). However, the relationship between saturation and days after slaughter – the shape value, α – differed significantly among the muscles (P < 0.001) (Table 10). TEND and RUMC had stronger curvature than BLD and KNUCK (clear from inspection of Figure 49, and α in Table 10). The highest saturations occurred after 13 to 19 days of aging (parameter β)

but did not differ significantly among muscles (Table 10). The highest saturation values between 36 and 37 were seen in RUMC, RUMH and TEND. By comparison, the remaining muscles had significantly lower maximum saturation values, ranging between 34 and 35 (parameter γ in Table 10).

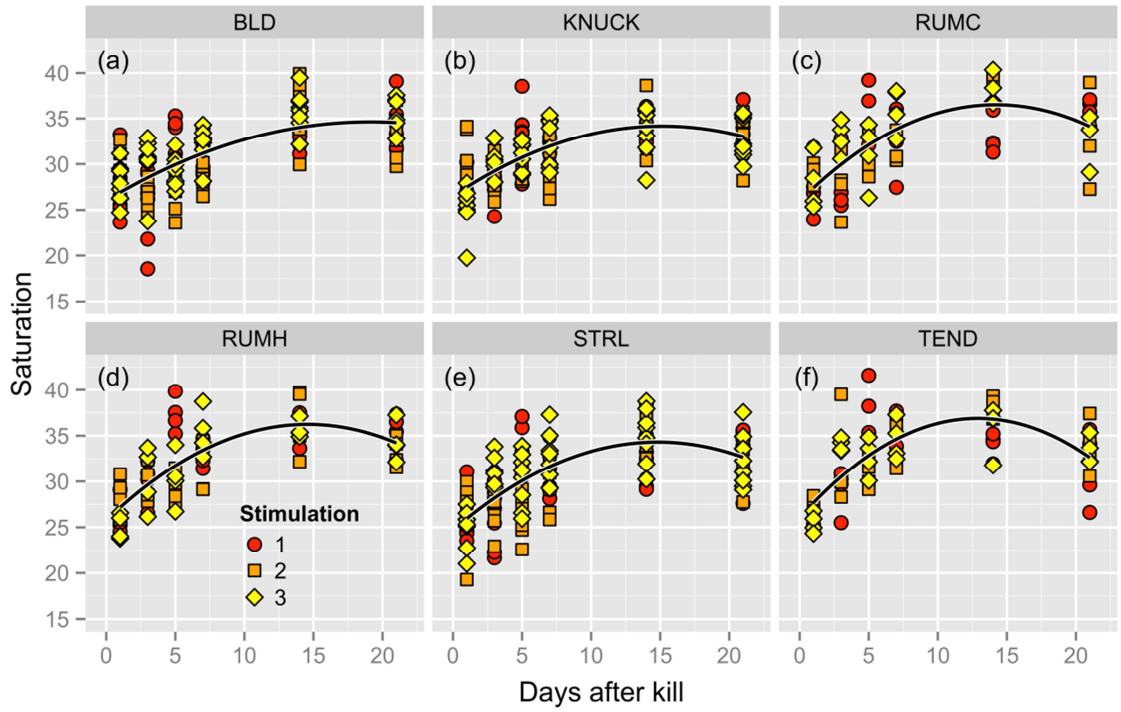

Figure 49. Colour intensity indicator saturation, as a function of days after slaughter by muscles (a – f) and stimulation treatment (red circles = Stimul1, orange squares = Stimul 2, yellow diamonds = Stimul 3). Lines represent fits from a nonlinear mixed-effects model with individual parameter estimates for each muscle based on the alternative quadratic function.

Table 10 Results from a pairwise-comparison procedure following a nonlinear mixed-effects model for saturation with separate parameter estimates for each muscle (values in brackets are standard errors of the parameter estimates). Model parameters: α = shape parameter, β = days after slaughter (x-axis) when saturation values reached a maximum, γ = maximum saturation. Different lower case letters indicate significant differences between parameter estimates at P < 0.05.

Muscle	Model parameters (SE)		
	α	β	γ
BLD	-0.023 (0.006) [a]	19.13 (2.20) [a]	34.63 (0.40) [a]
KNUCK	-0.033 (0.006) [ab]	15.16 (0.88) [a]	34.16 (0.40) [a]
RUMC	-0.052 (0.009) [bc]	14.22 (0.67) [a]	36.52 (0.58) [b]
RUMH	-0.049 (0.009) [abc]	14.58 (0.75) [a]	36.24 (0.57) [b]
STRL	-0.044 (0.006) [abc]	14.84 (0.64) [a]	34.31 (0.40) [a]
TEND	-0.065 (0.009) [c]	12.90 (0.42) [a]	36.88 (0.60) [b]

Effects of stimulation treatment, muscle and days after slaughter on hue angle

Hue angle is defined as arctan (b*/a*), and it is colour that is seen by the eye. In the case of red meat, higher hue angles indicate browner meat.

Since the functions of a* and b* were usually positively humped (Figure 58 and Figure 61 in Appendix), it was likely that plots of hue angle against days of ageing would approximate straight lines, and so it proved to be (Figure 50). The pattern of the relationship between hue angle and days after slaughter was similar across stimulation methods (Figure 50) (*deltaAIC* = 2.20). This favoured a model with one common time smoother across stimulation methods, but the intercepts of this relationship varied with stimulation method (P <0.001, Table 26 in Appendix). Stimul 1 and 3 shared a similar intercept at Day 0, differing only by 0.003, whereas Stimul 2 showed a significantly lower intercept, - 0.01. Thus, the Stimul 2 line was lower and was significantly distinct from the Stimul 1 and 3 line (P <0.001).

The hue angle development over time varied across the muscles (*deltaAIC* = 45.32) in favour of model with individual time smoothers for each muscle. The hue angle increased linearly in RUMC, RUMH and STRL muscles; and in a curvilinear fashion in TEND (Figure 50). Hue angle did not change significantly over time in BLD and showed a slightly undulating pattern in KNUCK (Figure 50).

The multiple comparison testing showed that RUMC, RMH and STRL shared the same linear pattern of hue angle development (see lower case letters in Table 26 in Appendix); also, the smoothing terms for KNUCK and TEND did not differ significantly.

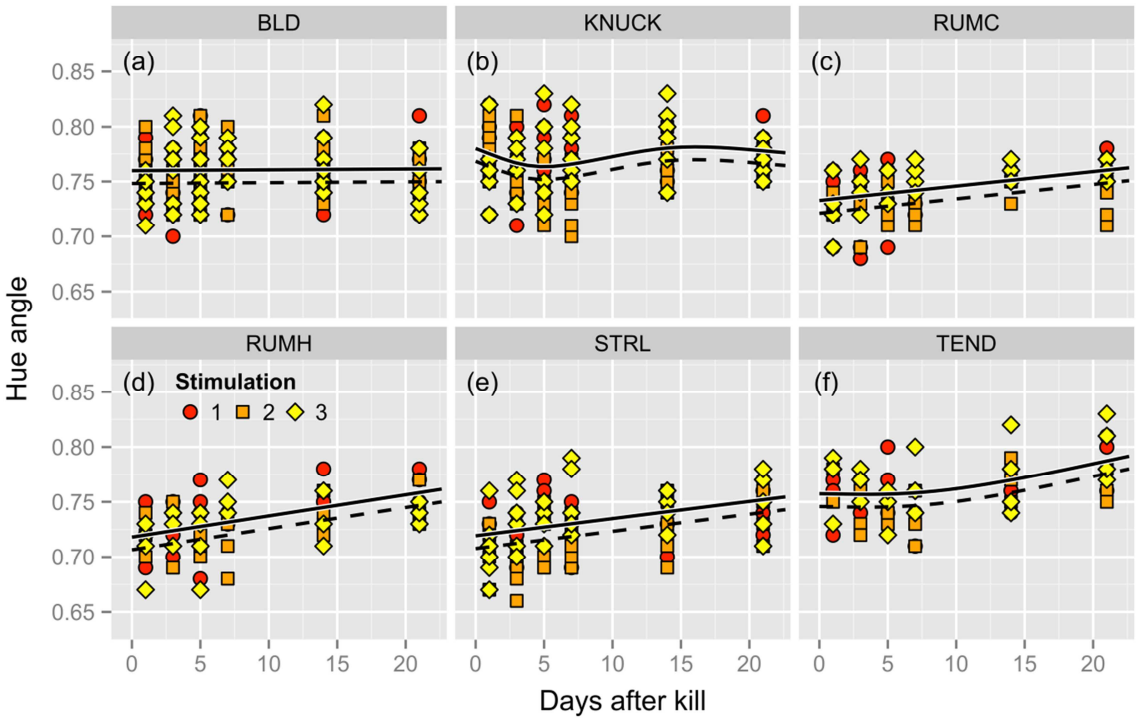

Figure 50 Hue angle as a function of days after slaughter by muscles (a – f) and stimulation (red circles = Stimul 1, orange squares = 2, yellow diamonds = 3). Lines represent smoother terms from a generalised additive mixed model (GAMM), solid lines = fits for the combined Stimul1 and 3, dashed lines = fits for Stimul2.

Effects of stimulation treatment, muscle and days after slaughter on Hunter L* and Japan colour assessment

Hunter L* is measure of percent total light reflectance – whatever the hue and intensity (saturation) of that light – and 'Japan' is a subjective assessment of colour using meat-coloured tiles as the reference.

The relationships between L* and time after slaughter, and the equivalent graphs for Japan showed some curvatures. Therefore for both parameters, a flexible generalised additive mixed model (GAMM) was used to test for differences between stimulation methods.

I will first discuss the reflectance L*, and later the Japan colour assessment.

For the data analysis of the reflectance indicator L*, a model comparison based on the difference in AIC between a model with a common smoother term and a model allowing smoothers for each stimulation method led strong support to the latter (deltaAIC = 20.23), indicating that the relationship between L* and days after slaughter varied with the stimulation method. Thus, the L* showed a linear trend with days after slaughter for Stimul1 and 3 (Figure 51), but not for Stimul2 (Figure 52).

In the Stimul1 and 3 analysis, the L* values showed a linear trend and therefore a linear mixed-effect model was used (Figure 51). Apart from muscle, none of the fixed terms was significant (Table 27 in Appendix). The Tukey contrasts tests showed that KNUCK had the highest L* values around 37, which was significantly brighter than the other muscles (Table 11; Figure 51). TEND and BLD showed similar values at around 33; whereas the lowest values of around 31 were seen in RUMC, RUMH and STRL.

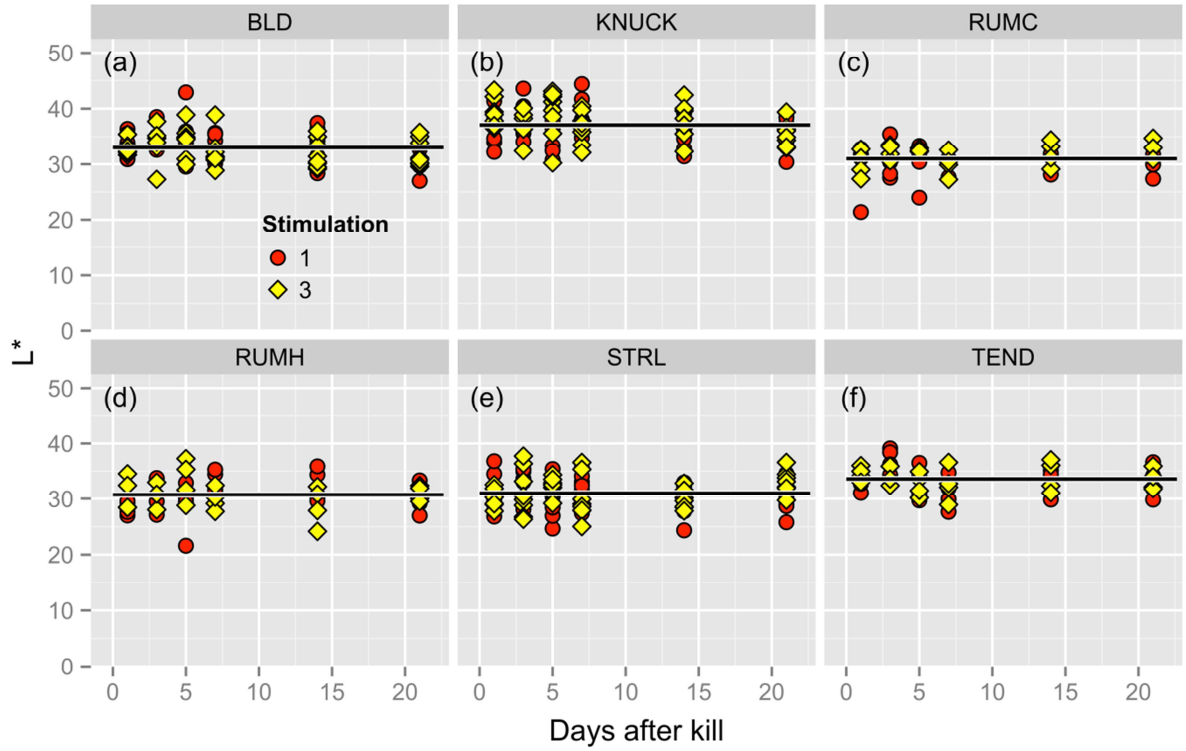

Figure 51 Light reflectance L* as a function of days after slaughter by muscles (a – f) and Stimul1 and 3 (red circles = Stimul1, yellow diamonds = 3). Lines represent fits from a linear mixed-effect model.

Table 11 Results from a multiple comparison on the estimates for muscle from a linear mixed-effects model for L* from Stimul1 and 3, where values in brackets are standard errors. Different lower case letters indicate significant differences between parameter estimates from a multiple-comparison procedure using Tukey contrasts.

Muscle	Model estimate (SE)
BLD	33.02 (0.46) [a]
KNUCK	37.08 (0.47) [b]
RUMC	31.00 (0.53) [c]
RUMH	30.73 (0.53) [c]
STRL	31.01 (0.46) [c]
TEND	33.59 (0.53) [a]

Stimul 2 data for L* were different from Stimul 1 and 3 data, as is clear from inspection of Figure 51 and Figure 52. Stimul 2 displayed an undulating response of L* over time, which was modelled using a generalised additive mixed model (GAMM) (Figure 52).

(A model comparison between a model with one common smoother term for all muscles in Stimul2 and a model allowing separate smoother terms for each muscle showed that a common smoother term was adequate (*deltaAIC* = 48.07). However, the curves varied considerably in their intercept across muscle (*deltaAIC* = 110.60 in favour of the model including a factor 'muscle'.)

A multiple comparison test with Tukey contrasts revealed that BLD and KNUCK, which had the largest L* value, differed significantly from all other muscles and from each other (Table 12; Figure 52). The remaining muscles did not differ significantly in their intercepts. Importantly, KNUCK was also the muscle in the Stimul1 and 3 analysis that showed the greatest L* value.

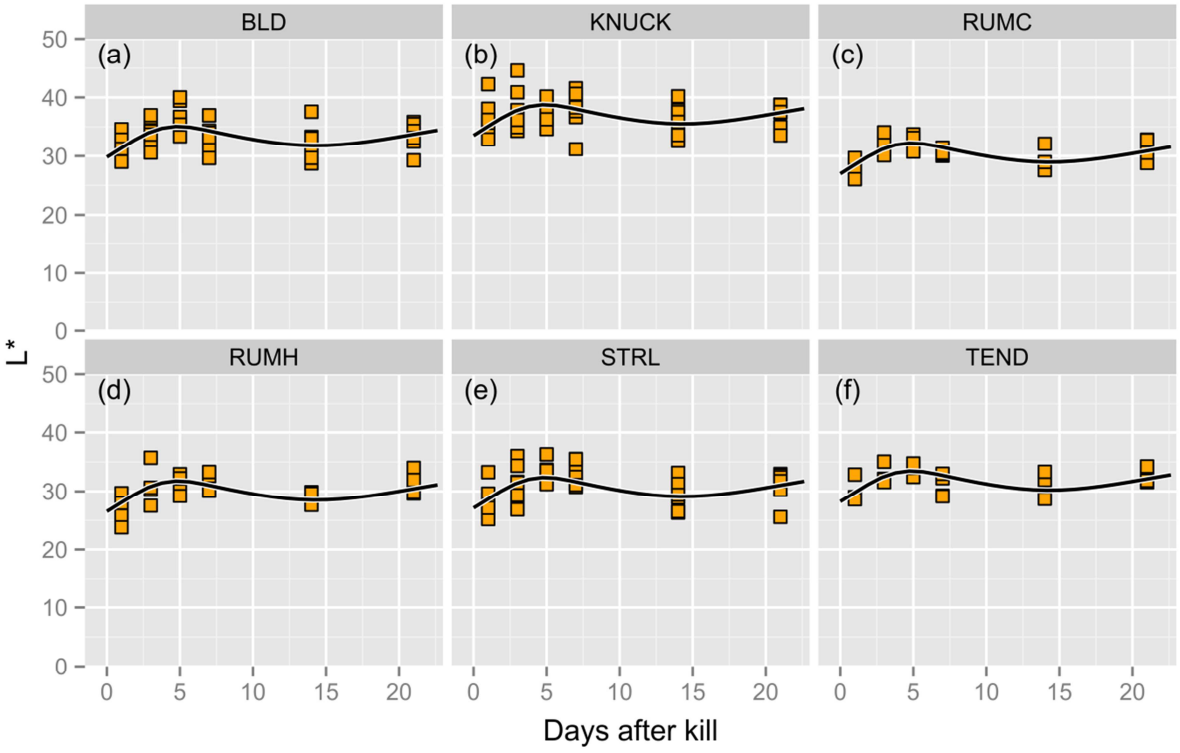

Figure 52 Light reflectance L* as a function of days after slaughter by muscles (a – f) for Simul 2. Lines represent smoother terms from a generalised additive mixed model (GAMM). Approximate significance of smooth terms: edf = 3.85, P < 0.001. edf = estimated degrees of freedom where an edf of 1 produces a straight lines, and an edf > 1 indicates curvature.

Table 12 Results from a multiple comparison on the estimates for muscle from a generalised additive mixed model of L* for Stimul 2 where values in brackets represent standard errors. Different lower case letters indicate significant differences between parameter estimates from a multiple-comparison procedure using Tukey contrasts.

Muscle	Model estimate (SE)
BLD	33.39 (0.45) [a]
KNUCK	37.10 (0.51) [b]
RUMC	30.56 (0.48) [c]
RUMH	30.12 (0.47) [c]
STRL	30.71 (0.41) [c]
TEND	31.81 (0.51) [c]

Now, we consider the subjective Japan colour score data. Model comparisons showed that the temporal development of the Japan colour score from Stimul 1 and 3 could be described with one common smoother (deltaAIC = 5.33), but Stimul 2 differed greatly from the other two methods (Stimul1 versus 2: deltaAIC = 19.88; Stimul 2 versus 3: deltaAIC = 11.30). Thus just as for L*, Japan colour score in Stimul1 and 3 data were pooled for further analysis and Stimul2 was treated separately.

Figure 53 and Figure 54 show the temporal plots for the two analyses. What is immediately obvious is a reciprocal relationship between these Japan plots and the equivalent L* plots (Figure 51 and Figure 52). This interesting relationship is discussed later. But for the present, the formal temporal analysis is described, just as for L* data.

The Japan colour score showed a linear trend over time for Stimul1 and 3 and therefore a linear mixed-effects model was used (Figure 53). None of the fixed terms was significant apart from muscle ($P < 0.001$, Table 28 in Appendix). RUMC, RUMH and STRL showed the highest Japan colour values at around 5.2 and were significantly higher than the values displayed by the other muscles (Figure 53; Table 13).

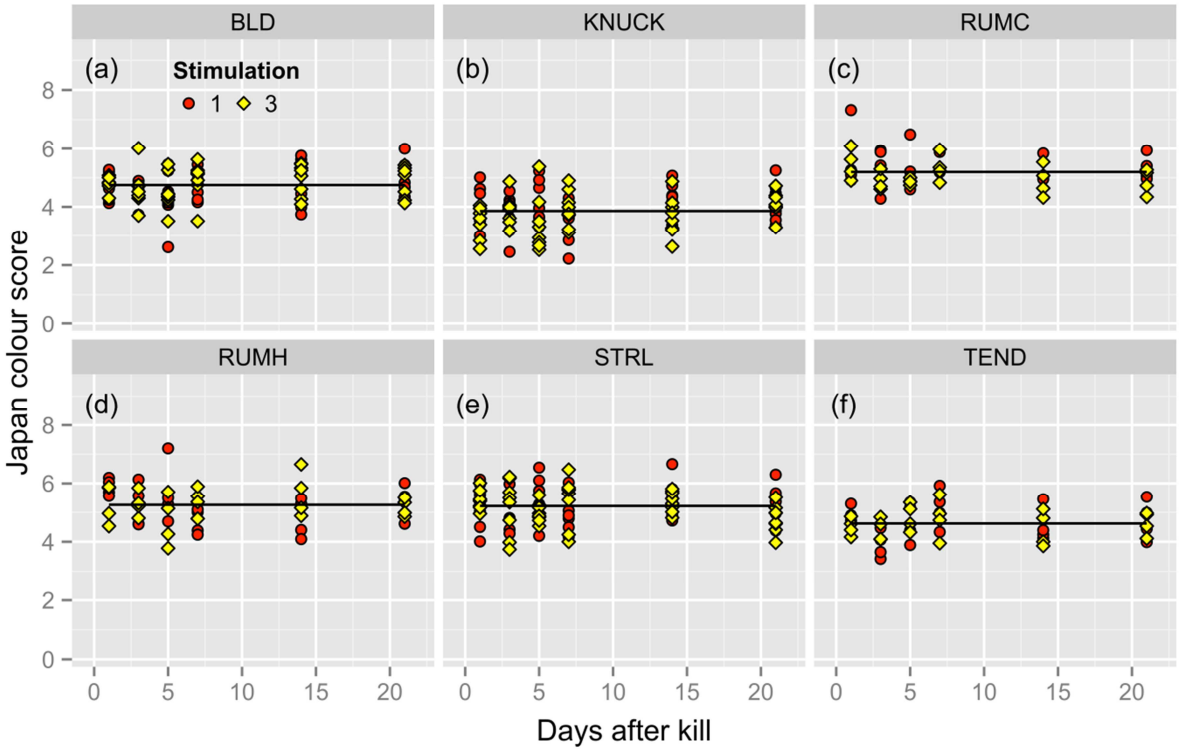

Figure 53 Japan colour score as a function of days after slaughter by muscles (a – f) and Stimul1 and 3 (red circles = Stimul1, yellow diamonds = 3). Lines represent fits from a linear mixed-effects model.

Table 13. Results from a multiple comparison on the estimates for muscle from a linear mixed-effects model for Japan data from Stimul1 and 3, where values in brackets are standard errors. Different lower case letters indicate significant differences between parameter estimates from a multiple-comparison procedure using Tukey contrasts.

Muscle	Model estimate (SE)
BLD	4.75 (0.10) [a]
KNUCK	3.86 (0.10) [b]
RUMC	5.20 (0.12) [c]
RUMH	5.25 (0.12) [c]
STRL	5.21 (0.10) [c]
TEND	4.62 (0.11) [a]

The muscles from Stimul 2 displayed an undulating response of Japan data over time, which was modelled using a generalised additive mixed model (Figure 54). All muscles attained a maximum value at about 14 days after slaughter. A model

comparison between a model with one common smoother term for all muscles and a model allowing separate smoother terms for each muscle showed that one common smoother term was adequate (deltaAIC = 43.21 in favour of the model with common smoother for muscles). However, the curves significantly varied in their intercept with muscle (deltaAIC = 173.28 in favour of the model including a factor ‘muscle’). Multiple comparison analysis with Tukey contrasts showed that BLD and KNUCK differed significantly from all other muscles and from each other (Table 14). The KNUCK muscle had the lowest Japan colour value, followed by BLD, TEND, STRL, RUMC and RUMH.

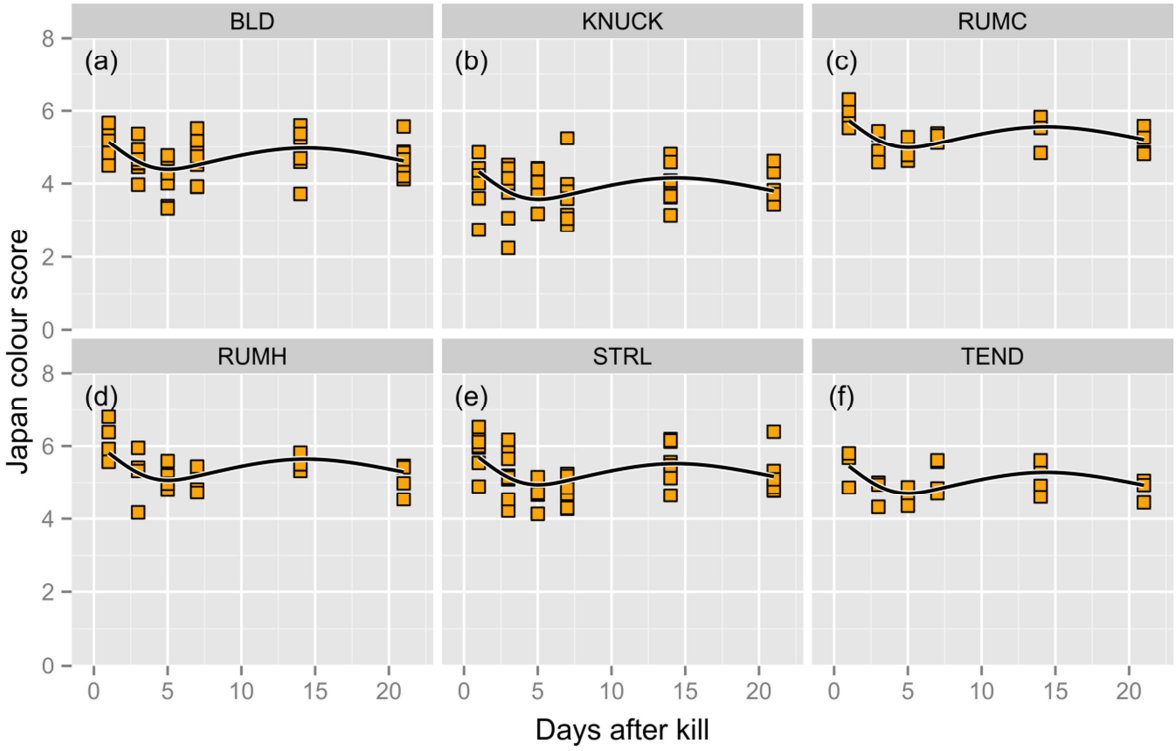

Figure 54 Japan colour score as a function of days after slaughter by muscles (a – f) for Stimul 2. Lines represent smoothing terms from a generalised additive mixed model (GAMM). Approximate significance of smooth terms: edf = 3.80, P < 0.001. edf = estimated degrees of freedom (edf of 1 produces a straight line, edf > 1 indicates curvature.

Table 14 Results from a multiple comparison on the estimates for muscle from a generalised additive mixed model of Japan colour score for Stimul 2 where values in brackets represent standard errors. Different lower case letters indicate significant differences between parameter estimates from a multiple-comparison procedure using Tukey contrasts.

Muscle	Model estimate (SE)
BLD	4.69 (0.09) [a]
KNUCK	3.88 (0.09) [b]
RUMC	5.29 (0.11) [c]
RUMH	5.38 (0.11) [c]
STRL	5.25 (0.09) [c]
TEND	5.01 (0.12) [ac]

Effects of stimulation treatment, muscle and days after slaughter on cooked meat toughness by shear force

The relationship between shear force and days after slaughter was similar across the stimulation methods (deltaAIC = 9.41) in favour of a model with one common time smoother across stimulation methods (Figure 55). However, the development of reduced shear force varied significantly across the muscles (deltaAIC = 45.32 in favour of a model with individual time smoothers for each muscle, Table 29 in Appedix). The shear force decreased in curvilinear or linear fashion in all muscles except TEND. This muscle showed a slight increase in shear force with time (Figure 55), but on average was the most tender muscle.

Multiple comparison testing showed that BLD and RUMC shared the same pattern of shear force development but all other patterns (smoothers) were significantly different from each other (top part of Table 29 in appendix). KNUCK and STRL showed the most curvature and their pattern, indicating that shear force reached a steady state between 7 and 14 days after slaughter.

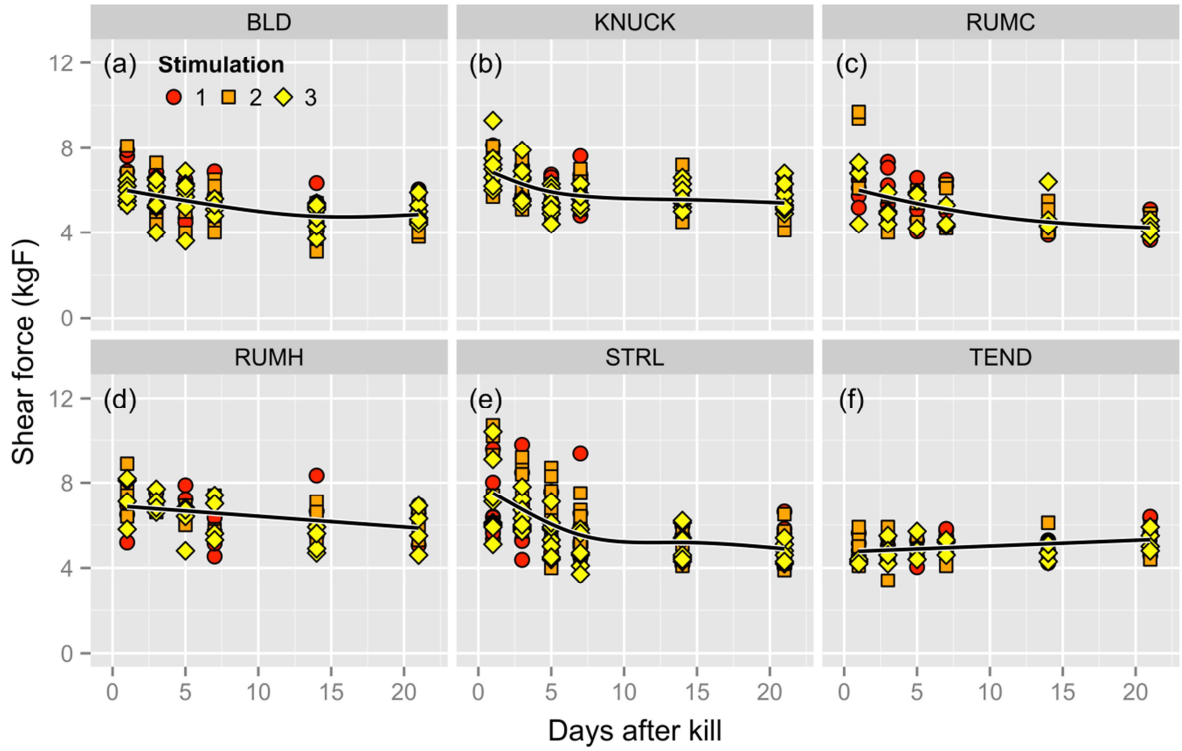

Figure 55 Meat shear force (kgF) as a function of days after slaughter by muscles (a – f) and stimulation method (red circles = Stimul 1, orange squares = 2, yellow diamonds = 3). Lines represent smoother terms from a generalised additive mixed model

Discussion

The discussion for **Chapter 5** on various meat quality attributes is divided into four subsections: drip loss; meat colour described by a*, b* and their derivative values; L* value and Japan colour; and cooked meat shear force.

Drip loss

Based on the pH-temperature decline curves (Figure 29), it was expected that Stimul 1 was the most likely to generate heat shortening, and therefore higher drip loss %. However, this was not the case. So, we assume that the extent of heat shortening from Stimul 1 was minimal and insufficient to denature the myofibrillar proteins of the muscles.

With the data pooled across stimulation treatments, there were some significant differences between muscles, but also some non-significant differences. These non-significant differences were experienced with 36 animals, but from thousands of animals, these subtle differences can become commercially important. Therefore from a commercial perspective, the order of maximum drip loss - described by the asymptotic values - is important and irrespective of statistical significance: *Gluteus medius* (RUMH, highest percent drip loss) > *Gluteobiceps* (RUMC) > *Psoas major* (TEND) > *Rectus femoris* (KNUCK) > *Longissimus dorsi* (STRL)> *Infraspinatus* (BLD, lowest percent drip loss).

Meat colour described by derivatives of a and b* values*

Positive Hunter a* and b* values represent redness and yellowness, respectively, and are combined in the functions $\sqrt{(a^{*2} + b^{*2})}$, termed saturation, which represents colour intensity. The arctan (b*/a*): hue angle, is the colour that the eye sees. Hunter values a* and b* alone are not commercially important.

The Hunter a* and b* values showed asymptotic or more commonly humped curves (refer to Figure 58 and Figure 61 in Appendix), thus the saturation values were also humped. Saturation is a measure of colour intensity and Figure 49 showed that the peak saturation occurred between about 13 and 19 days after slaughter.

The stimulation method had no significant effect on saturation values. RUMC, RUMH and TEND had significantly higher saturation values than the other three muscles

(P< 0.05). Irrespective of statistical significance, at 15th day from slaughter, the descending order of maximum saturation in Table **16** was: *Psoas major* (TEND, most saturated), *gluteobiceps* (RUMC) > *gluteus medius* (RUMH) > *infraspinatus* (BLD) > *longissimus dorsi* (STRL) > *rectus femoris* (KNUCK, least saturated).

For red meat, a higher hue angle value indicates browner meat. Although the maximum value occurred at 21 days, we would expect the values will continue to increase over time. This is evident in Figure 50, where the hue angle tended to increase with time. Stimul 1 and 3 pooled had slightly higher hue values than Stimul 2, but the difference may be undetectable by eye (Figure 50). The brownest muscle at 21 days was *psoas major*.

Browning is commercially undesirable for product appearance. 13 and 19 days after slaughter showed the highest saturation and least hue angle, which suggested that the meat within this period would present the most desirable colour.

Meat colour described by L* and Japan colour

For Stimul 1 and 3 pooled, L* was unvarying with time, so 13 to 19 days after slaughter appears to be optimum for those stimulation treatments. For Stimul 2, the L* values undulate. But, when Figure 51 and Figure 52 were scaled identically, the undulations would appear much less important.

The Japan colour data was also best analysed in two groups, similar as L* values. Stimul 1 and 3 were pooled together, and Stimul 2 separately. What was immediately striking was an apparent reciprocal relationship between L* and Japan colour within the two analytical groups, where the peak values of the variables were plotted near perfect inverse relationships (Figure 56 and Figure 57). It could be argued that L* values would give an objective value of L* – and inversely Japan colour – but given the near perfect relationships described, it could be argued that colour tiles (Figure 23) were as good, certainly cheaper and more reliable by virtue of their simplicity.

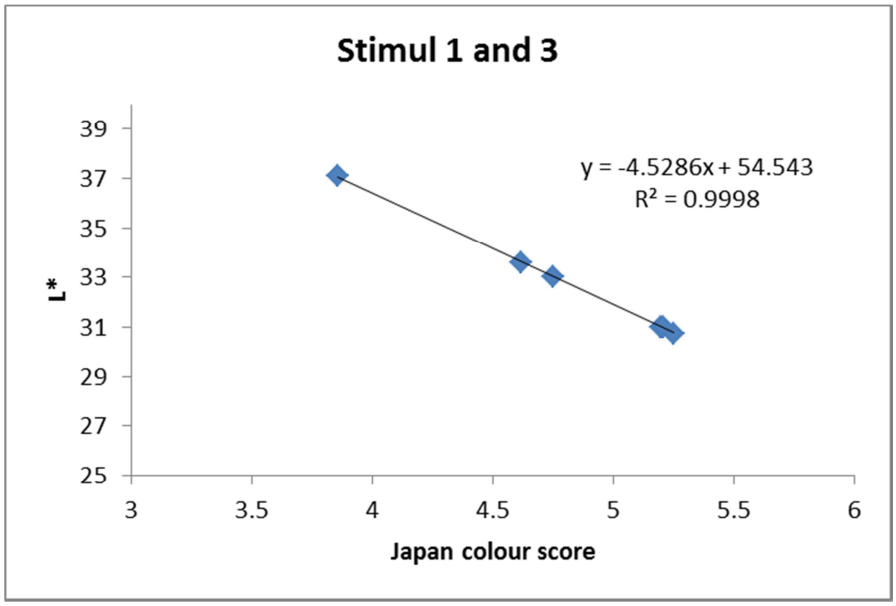

Figure 56 Inverse relationship between L* and Japan colour score for Stimul 1 and 3.

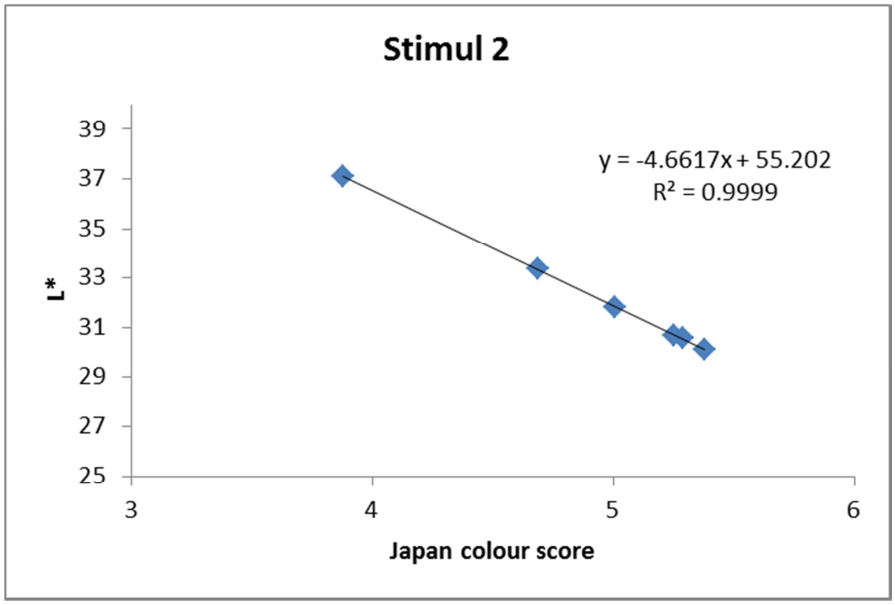

Figure 57. Inverse relationship between L* and Japan colour score for Stimul 2

Cooked meat shear force

There is no significant difference on the stimulation methods for shear force.

The initial shear force values for *infraspinatus* (BLD), *rectus femoris* (KNUCK) *gluteobiceps* (RUMC), *gluteus medius* (RUMH) and *psoas major* (TEND) were below 10 kgF, and would provide an acceptable eating experience even one day after slaughter. The initially low value of around 4.8 kgF for psoas major was not surprising. The muscle's location and its function in the live animal guarantee its immediate tenderness. The *longissimus dorsi* (STRL) showed a high variation of

tenderness (between 5 kgF to 12 kgF): initially meaning that some STRL muscles would be unacceptably tough at 1 day after slaughter. In this respect, STRL was the most variable muscle for tenderness, and that variability was maintained to 7 days after slaughter. The tenderness variability of all muscles decrease with time and that is consistent with common experience. All muscles would provide a good eating experience a week to 10 days after slaughter.

Results summary:

The results were summarised in the following tables:

Table 15 Mean drip loss and cooked meat shear force of muscles ranked at key days after slaughter

	Drip loss				Cooked meat shear force		
	Day 1	Day 15	Day 21		Day 1	Day 15	Day 21
Most drip	Not applicable	*Gluteus medius*	*Gluteus medius*	Toughest	*Longissimus dorsi*	*Gluteus medius*	*Gluteus medius*
	Not applicable	*Psoas major*	*Gluteobiceps*		*Rectus femoris*	*Rectus femoris*	*Rectus femoris*
	Not applicable	*Gluteobiceps*	*Psoas major*		*Gluteus medius*	*Longissimus dorsi*	*Psoas major*
	Not applicable	*Rectus femoris*	*Rectus femoris*		*Gluteobiceps =*	*Psoas major*	*Infraspinatus =*
	Not applicable	*Longissimus dorsi*	*Longissimus dorsi*		*=Infraspinatus*	*Infraspinatus*	*=Longissimus dorsi*
Least drip	Not applicable	*Infraspinatus*	*Infraspinatus*	Tenderest	*Psoas major*	*Gluteobiceps*	*Gluteobiceps*

Table 16 Mean saturation and hue of muscles ranked at key days after slaughter

	Saturation				Hue		
	Day 1	Day 15	Day 21		Day 1	Day 15	Day 21
Most colour	*Psoas major=*	*Psoas major=*	*Gluteus medius*	Most brown	*Rectus femoris*	*Rectus femoris*	*Psoas major*
	=Gluteobiceps	*=Gluteobiceps*	*Gluteobiceps*		*Infraspinatus*	*Psoas major*	*Rectus femoris*
	=Gluteus medius	*=Gluteus medius*	*Infraspinatus*		*Psoas major*	*Infraspinatus*	*Infraspinatus*
	=Rectus femoris	*Infraspinatus=*	*Psoas major*		*Gluteobiceps*	*Gluteobiceps*	*Gluteobiceps*
	=Infraspinatus	*=Longissimus dorsi*	*Longissimus dorsi*		*Gluteus medius*	*Gluteus medius*	*Gluteus medius*
Least colour	*=Longissimus dorsi*	*=Rectus femoris*	*Rectus femoris*	Least brown	*Longissimus dorsi*	*Longissimus dorsi*	*Longissimus dorsi*

Table 17 Mean lightness of muscles ranked at key days after slaughter

	Lightness L* (Stimul 1&3)				Lightness L* (Stimul 2)		
L*	Day 1	Day 15	Day 21	L*	Day 1	Day 15	Day 21
Lightest	*Rectus femoris*	*Rectus femoris*	*Rectus femoris*	Lightest	*Rectus femoris*	*Rectus femoris*	*Rectus femoris*
	Psoas major=	*Psoas major=*	*Psoas major=*		*Infraspinatus*	*Infraspinatus*	*Infraspinatus*
	=Infraspinatus	*=Infraspinatus*	*=Infraspinatus*		*Psoas major*	*Psoas major*	*Psoas major*
	Longissimus dorsi	*Longissimus dorsi*	*Longissimus dorsi*		*Longissimus dorsi*	*Longissimus dorsi*	*Longissimus dorsi*
	=Gluteobiceps	*=Gluteobiceps*	*=Gluteobiceps*		*=Gluteobiceps*	*=Gluteobiceps*	*=Gluteobiceps*
Darkest	*=Gluteus medius*	*=Gluteus medius*	*=Gluteus medius*	Darkest	*=Gluteus medius*	*=Gluteus medius*	*=Gluteus medius*

Table 18 Mean lightness and Japan colour of muscles ranked at key days after slaughter

	Japan colour score (Stimul 1&3)				Japan colour score (Stimul 2)		
Japan	Day 1	Day 15	Day 21	Japan	Day 1	Day 15	Day 21
Most colour	*Gluteus medius=*	*Gluteus medius=*	*Gluteus medius=*	Most colour	*Gluteus medius=*	*Gluteus medius=*	*Gluteus medius=*
	=Longissimus dorsi	*=Longissimus dorsi*	*=Longissimus dorsi*		*=Gluteobiceps*	*=Gluteobiceps*	*=Gluteobiceps*
	=Gluteobiceps	*=Gluteobiceps*	*=Gluteobiceps*		*=Longissimus dorsi*	*=Longissimus dorsi*	*=Longissimus dorsi*
	Infraspinatus=	*Infraspinatus=*	*Infraspinatus=*		*Psoas major*	*Psoas major*	*Psoas major*
	=Psoas major	*=Psoas major*	*=Psoas major*		*Infraspinatus*	*Infraspinatus*	*Infraspinatus*
Least colour	*=Rectus femoris*	*=Rectus femoris*	*=Rectus femoris*	Least colour	*Rectus femoris*	*Rectus femoris*	*Rectus femoris*

Chapter 6
Concluding Discussion

Chapter 4 and Chapter 5 reported the results and discussion for the two parts of this project, where bovine carcasses were subjected to three electrical treatments after slaughter.

The first part (Chapter 4) reported on the pH-temperature profiles in two indictor muscles, *longissimus dorsi* and *semimembranosus*, and the required outcome was a decision on which electrical stimulation treatments was the best in terms of meat quality. This was of much concern to Silver Fern Farms because the traditional 15Hz immobilisation proved to accelerate glyoclysis which results in a faster pH decline at an earlier time, while the meat temperature is high; and poses heat shortening, especially on the deep muscles like *Semimembranosus*. The high 400Hz did accelerate some glycolysis, which is sufficient to avoid cold shortening window and not fall into heat shortening. However, with 400Hz applied at immobilisation, the setting results in higher incidence of burst tenderloins and ecchymosis, more commonly known as blood splash in the industry. These issues came to light in many beef processing plants after upgrading to 400Hz. Therefore, a mixture of 15Hz and 400Hz was used to replace 400Hz.

The three stimulation treatments chosen here were:

Stimul 1 was a continous 15 Hz immobilisation for 40 seconds

Stimul 2 was 4 seconds of 15 Hz, followed by 36 seconds of 400 Hz

Stimul 3 comprised Stimul2 plus Smart Stimulation at 30 minutes post slaughter

Although the data were unfortunately limited for Stimul 1, historical data strongly suggested that there would be a severe risk of heat shortening from that treatment, particularly in the deeper *semimembranous* muscle.

By contrast, Stimul 2 and 3 showed little tendency for heat shortening. The contemporary data also showed that carcass weight had no effect on the time temperature profiles, perhaps unexpected because in theory larger carcasses should cool more slowly, which will accelerate glycolysis and pH decline. As reported earlier, the 15 Hz and 400 Hz combination in Stimul 2 reduces the incidence of burst

tenderloins and ecchymosis. Also, Stimul 2 is an inexpensive, easy-to-operate and worker-safe electrical treatment. The 15 Hz stimulation (Stimul 1) is useful for accelerating pH decline, but needs to be controlled to prevent excessive pH fall; 40 seconds clearly being too long.

Stimul 3 requires application of SmartStim, after application of Stimul 2. Its installation in an abattoir adds cost and the question is: is it worth in terms of the temperature-pH-time profiles and their variability between carcasses? The pH-time graphs in Chapter 4 answer this question for the two indicator muscles, and the meat quality data in Chapter 5 answer this question over a wider range of muscles.

I will consider the indicator muscles first. For *longissimus dorsi*, Stimul 1 had the most variable pH values, particularly at early times after slaughter. Clearly this could result in more variable meat quality. Variability was reduced in Stimul 2 and – especially - Stimul 3 (Smart Stimulation). Thus from these data, Stimul 3 appears more attractive. Carne Technology claims that the Smart Stimulation (Stimul 3) can control the rate of pH fall and ultimate pH prediction. Stimul 3 did demonstrate an ability to control pH decline rate in the first few hours but only in *longissimus dorsi*. However, any advantage of reduced variability did not extend to *semimembranosus*. This is possibly because the SmartStim stimulation bar is applied only to *longissimus dorsi* and the contact time is very short, i.e. <15 seconds when compared to the alternative Smart Stimulation tunnel, about 80 seconds. Outside the scope of this project, I performed another pH and temperature window assessment in the Auckland Meat Processor abattoir. The immobilisation settings at AMP have a short period (2 seconds) of 15 Hz, followed by about 18 seconds of continuous high frequency. The immobilisation setup is similar to that at Silver Fern Farms (15 Hz + 400 Hz), but the 15 Hz duration is shorter by 87%. Their Smart Stimulation tunnel is located after grading and before chiller entry. When observed, each carcass side spent about 80 seconds in the tunnel and the stimulation stops when desired pH is attained. The results from AMP are very similar to those for Stimul 3, where the Smart Stimulation is likely to yield more consistent meat eating quality in *longissimus dorsi* and possibly proximate muscles.

Consider now the results in Chapter 5, where we evaluate the effect of the three stimulation treatments on meat quality in six indicator muscles at various days after slaughter. Stimulation treatment had no effect on drip loss, but the data reveal the

rank order of drip loss amongst muscles is the same as the other meat quality variables shown in the Results summary: Table 15, Table 16, Table 17 and Table 18. Stimulation had no effect on saturation, but hue angle was lower (less brown) in Stimul 2, however whether this could be detected by eye is unknown.

This study did not have a non-stimulated control group, but provides a comparison between three stimulation systems. This explains why there was few significant differences in meat quality attributes due to stimulation systems, as any of the stimulation system can improve meat quality. The current work is aimed to choose which stimulation system could benefit the company the most in meat quality consistency, operating cost and any added objective measurement system.

The question about the efficacy of Smart Stimulation (Stimul 3) can now be answered. In terms of meat quality, it appears to give minimal advantage over a Stimul 2-type protocol. In hindsight, the meat processor could choose an inexpensive Stimul 2 protocol and tailor the duration of the15 Hz and 400 Hz frequencies for process optimisation, in terms of pH-temperature profile. On a separate trial, I have been working with Carne Technology on ultimate pH prediction work on beef and lamb, but with little success in the last few years. At present, the company has yet to get a significant commercial benefit from Smart Stimulation. So Stimul 2 is currently in practice for all Silver Fern Farms beef processing plant.

On a more positive note, the results on Table 15, Table 16, Table 17 and Table 18 are useful contribution for Silver Ferns Farm's portion size product development project. As a general rule, the muscles that were studied in this project will reach an optimum tenderness and colour at Day 15. However, a larger data set will be required for validity.

As for future work, there are some areas of interest that could be further studied. On a separate visit to another meat processor, I was under the impression that most beef plants in New Zealand are operating under an alternating frequency (15 Hz + >300 Hz) during immobilisation. It could be of interest to understand the myology and muscle contraction % of beef muscle under the high frequency stimulation system, particularly >300Hz. The incidence of blood splash is historically associated with animal handling practice and delay in bleeding, however on this study, we believe

that the high frequency immobilisation could be the factor and no one has done such study.

The remarkably precise inverse relationship between the Japan colour score and L* means that colour tiles were as good, certainly cheaper and more reliable by virtue of their simplicity. This colour grading technique is currently adapted by Silver Fern Farms Beef^{EQ} grading system.

If this project were to be repeated, a number of recommendations can be made. Firstly, a larger scale study using an unvarying electrical input profile across multiple abattoir to create a better muscle profile database. Secondly, the carcass selection criteria could be modified to include marbling, ossification and hump height, which are also contributors to consumer eating experience.

References

AMSA. (2001). AMSA Meat Color Measurement Guidelines Measurement. In *Proceedings of the 44th Annual Reciprocal Meat Conference*. Kansas State Univeristy, Manhattan, Kansas.

Aston, R. (1991). *Principles of biomedical instrumentation and measurement*.

Beef and Lamb NZ (2010) *Beef and Lamb information.* Retrived from http://www.beeflambnz.com/Global/Microsite/index.html

Bendall, J. R. (1978). Variability in rates of pH fall and of lactate production in the muscles on cooling beef carcasses. *Meat Science*, *2*(2), 91–104. doi:10.1016/0309-1740(78)90010-4

Bouton, P. E., Ford, A. L., Harris, P. V, & Shaw, F. D. (1980). Electrical stimulation of beef sides. *Meat Science*, *4*(2), 145–55. doi:10.1016/0309-1740(80)90038-8

Carse, W. A. (1973). Meat quality and the acceleration of post-mortem glycolysis by electrical stimulation. *International Journal of Food Science & Technology*, *8*(2), 163–166. doi:10.1111/j.1365-2621.1973.tb01702.x

Chiang, W. ., Strasburg, G. M. ., & Byrem, T. M. (2007). *Food Chemistry: Principles and Applications*. (Y. H. Hui, Ed.) (2nd Editio.). Science technology System, West Sacramento, CA.

Chrystall, B. B., & Devine, C. E. (1978). Electrical stimulation, muscle tension and glycolysis in bovine Sternomandibularis. *Meat Science*, *2*(1), 49–58. doi:10.1016/0309-1740(78)90021-9

Collins, F. V. (1954). *Meat Inspection in Australia*. South Australian School of Mines & Industries, Adelaide.

CSIRO. (2006, February). Electrical inputs during processing. *Meat Technology Update*, 1–4. Retrieved from http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_06-1.pdf

Devine, C. E., Ellery, S., & Averill, S. (1984). Responses of different types of ox muscle to electrical stimulation. *Meat Science*, *10*(1), 35–51. doi:10.1016/0309-1740(84)90030-5

Devine, C., Hopkins, D., Hwang, I., Ferguson, D. M. ., & Richards, I. (2004). Electrical stimulation. In W. Jensen, C. Devine, & M. Dikeman (Eds.), *Encyclopedia of meat sciences* (pp. 413–423).

Dransfield, E. (1994). Optimisation of tenderisation, ageing and tenderness. *Meat Science*, *36*(1-2), 105–21. doi:10.1016/0309-1740(94)90037-X

Dulhunty, A. F. ., Haarmann, C. S. ., Green, D. ., Laver, D. R. ., Board, P. G. ., & Casarotto, M. G. (2002). Interaction between dihydropyridine receptors and ryanodine receptors in striated muscle. *Prog BiophysMol Bio*, *79*, 45–75.

Farm IQ (2010) *From Plate to Pasture.* Retrieved from http://www.farmiq.co.nz/sites/default/files/FarmIQ-PGP-Business-Plan-Summary.pdf

Geesink, G. H., Bekhit, A. D., & Bickerstaffe, R. (2000). Rigor temperature and meat quality characteristics of lamb longissimus muscle. *J Anim Sci*, *78*(11), 2842–2848. Retrieved from http://www.journalofanimalscience.org/content/78/11/2842.short

Gilbert, K. V., Davey, C. L., & Newton, K. G. (1977). Electrical stimulation and the hot boning of beef. *New Zealand Journal of Agricultural Research*, *20*(2), 139–143. doi:10.1080/00288233.1977.10427317

Goll, D. E. ., Thompson, V. F. ., Li, H., Wei, W. ., & Cong, J. (2002). The calpain system. *Physiol Rev*, (83), 731–801.

Harsham, A., & Deatherage, E. (1951, March 13). Tenderization of meat. USA. Retrieved from http://www.google.com/patents/US2544681

Huff-Lonergan, E. T. ., Mitsuhashi, T. ., Beekman, D. D., Parrish, F. C. ., Olson, D. G. ., & Robson, R. M. (1996). Proteolysis of specific muscle structural proteins by u-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. *Journal of Animal Science*, *74*, 993–1008.

Hunt, M. C., & Hedrick, H. B. (1977). PROFILE OF FIBER TYPES AND RELATED PROPERTIES OF FIVE BOVINE MUSCLES. *Journal of Food Science*, *42*(2), 513–517. doi:10.1111/j.1365-2621.1977.tb01535.x

Huxley, A. F. ., & Hanson, J. (1954). Changes in the cross striations of muscles during contraction and stretch and their structural interpretation. *Nature*, *173*, 973–976.

Hwang, I. H., Devine, C. E., & Hopkins, D. L. (2003). The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. *Meat Science*, *65*(2), 677–91. doi:10.1016/S0309-1740(02)00271-1

Hwang, I. H., & Thompson, J. M. (2002). A technique to quantify the extent of postmortem degradation of meat ultrastructure. *ASIAN AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES*, *15*(1), 111–117.

Kastner, C. L., Schwenke, J. R., Kenney, P. B., Campbell, R. E., Kendall, J. A., & Milliken, G. A. (1993). Comparisons of the effect of electrical stimulation methods on postmortem pH decline in beef muscle. *Meat Science*, *35*(2), 183–90. doi:10.1016/0309-1740(93)90048-M

KOOHMARAIE, M., BABIKER, A. S., MERKEL, R. A., & DUTSON, T. R. (1988). Role of Ca ++ -Dependent Proteases and Lysosomal Enyzmes in Postmortem Changes in Bovine Skeletal Muscle. *Journal of Food Science*, *53*(5), 1253–1257. doi:10.1111/j.1365-2621.1988.tb09251.x

Lawrie, R. A. ., & Ledward, D. A. (2006). *Lawrie's Meat Science* (7th ed.). Woodhead Publishing Limited.

Locker, R. H., & Hagyard, C. J. (1963). A cold shortening effect in beef muscles. *Journal of the Science of Food and Agriculture*, *14*(11), 787–793. doi:10.1002/jsfa.2740141103

Lopez, C., & Herbert, E. W. (1975). *The private Franklin: The Man and his family* (1st ed., p. 44). W.W. Norton and Company, New York.

MacIntosh, B. R. ., Gardiner, P. ., & McComas, A. J. (2005). *Skeletal muscle: Form and Function*. Human Kinetics Publishers, Champaign, IL.

Marsh, B. B., & Thompson, J. F. (1958). Rigor mortis and thawrigor in lamb. *Journal of the Science of Food and Agriculture*, *9*(7), 417–424. doi:10.1002/jsfa.2740090707

Meat & Livestock Australia Ltd. (2010). *Meat Standards Australia beef information kit* (p. 27). North Sydney.

Offer, G. (1991). Modelling of the formation of pale, soft and exudative meat: Effects of chilling regime and rate and extent of glycolysis. *Meat Science*, *30*(2), 157–84. doi:10.1016/0309-1740(91)90005-B

Pearce, K. L., Hopkins, D. L., Williams, A., Jacob, R. H., Pethick, D. W., & Phillips, J. K. (2009). Alternating frequency to increase the response to stimulation from medium voltage electrical stimulation and the effect on objective meat quality. *Meat Science*, *81*(1), 188–95. doi:10.1016/j.meatsci.2008.07.018

Pearson, A. M. ., & Young, R. B. (1989). *Muscle and Meat Biochemistry*. Academic Press, Inc.. San Diego, CA.

POLIDORI, P., KAUFFMAN, R. G., & VALFRE, F. (1996). The effects of electrical stimulation on meat quality. *Italian Journal of Food Science*, *8*(3), 183–199. Retrieved from http://cat.inist.fr/?aModele=afficheN&cpsidt=3247216

Raymen, I. ., & Holden, H. M. (1994). The three-dimensional structure of a molecular motor. *TIBS*, *19*, 129–135.

Rossi, A. E. ., & Dirksen, R. T. (2006). Sacroplasmic reticulum: The dynamic calcium governor of muscle. *Muscle Nerve*, *33*, 715–731.

SAVELL, J. W., DUTSON, T. R., SMITH, G. C., & CARPENTER, Z. L. (1978). STRUCTURAL CHANGES IN ELECTRICALLY STIMULATED BEEF MUSCLE. *Journal of Food Science*, *43*(5), 1606–1607. doi:10.1111/j.1365-2621.1978.tb02553.x

Silver Fern Farms. (2012). *Annual report* (p. 35). Retrieved from http://www.silverfernfarms.com/assets/investors/silver-fern-farms-annual-report-2012.pdf

Silver Fern Farms. (2013). *Annual Report 2013* (p. 72). Retrieved from http://www.silverfernfarms.com/assets/investors/Silver-Fern-Farms-annual-report-2013.pdf

Simmons, N. J., Daly, C. C., Cummings, T. L., Morgan, S. K., Johnson, N. V, & Lombard, a. (2008). Reassessing the principles of electrical stimulation. *Meat Science*, *80*(1), 110–22. doi:10.1016/j.meatsci.2008.05.006

Simmons, N. J., Daly, C. C., Mudford, C. R., Richards, I., Jarvis, G., & Pleiter, H. (2006). Integrated technologies to enhance meat quality - An Australasian perspective. *Meat Science*, *74*(1), 172–9. doi:10.1016/j.meatsci.2006.05.007

Smith, G. C. (1985). Effects of electrical stimulation on meat quality, colour, grade. heat ring and palatability. In A. M. . Pearson & T. R. Dutson (Eds.), *Advances in Meat Research: Electrical Stimulation*. AVI, Westport, Connecticut.

Spangenburg, E. E. ., & Booth, F. W. (2003). Molecular regulation of individual skeletal muscle fiber types. *Acta Phsiol Scand*, (178), 413–424.

Strasburg, G., Xiong, Y. L. ., & Chiang, W. (2008). Physiology and Chemistry of Edible Muscle Tissues. In S. Damodaran, K. L. . Parkin, & O. R. Fennema (Eds.), *Fenemma's Food Chemistry* (4th ed., pp. 928–968). CRC Press.

Takahashi, G., Lochnert, J. V, & Marsh, B. B. (1984). Effects of low-frequency electrical stimulation on beef tenderness. *Meat Science*, *11*(3), 207–25. doi:10.1016/0309-1740(84)90038-X

Takahashi, G., Wang, S. M., Lochner, J. V, & Marsh, B. B. (1987). Effects of 2-Hz and 60-Hz electrical stimulation on the microstructure of beef. *Meat Science*, *19*(1), 65–76. doi:10.1016/0309-1740(87)90100-8

Tortoa, G. J. ., & Derrickson, B. (2006). *Principles of Anatomy and Physiology*. John Wiley and Sons, Inc., Hoboken, N.J.

Warris, P. D. (2000). *Meat Science: An Introductory Text*. CAB International.

Young, O. A. (1984). The biochemical basis of fibre types in bovine muscle. *Meat Science*, *11*(2), 123–37. doi:10.1016/0309-1740(84)90010-X

Appendix

The colour

The redness indicator a* varied significantly with electrical stimulation condition during the days after slaughter (P < 0.001) (Figure 58). All stimulation treatments had similar maxima around 25. While the Stimul 1 and 3 levelled to a plateau, Stimul 2 showed a terminal decline, which suggested that a quadratic model would be more useful. In Stimul 2, the initial response (R0 = 19.48) was significantly higher and the rate constant (*lrc* = -2.17) was significantly lower than Stimul 1 and 3. Stimul 1 and 3 shared similar estimates for both parameters (Table 19). Therefore, 1 and 3 were pooled for further analysis using an asymptotic model, whereas Stimul 2, which showed a more humped response was treated separately using an alternative quadratic model.

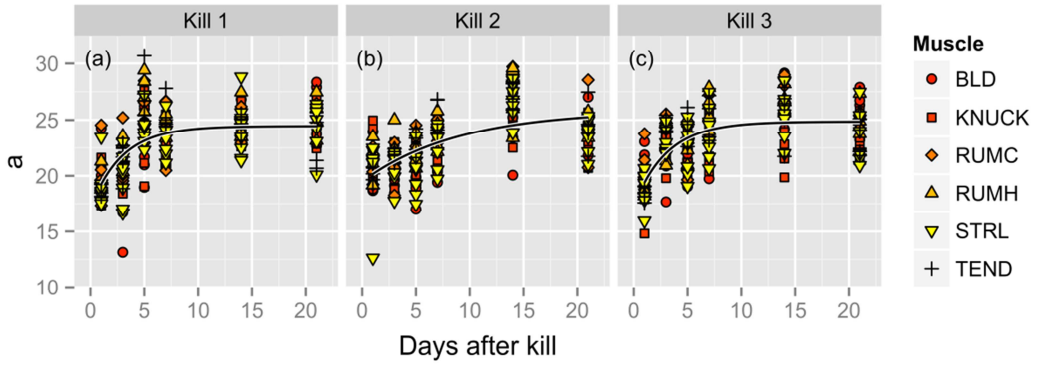

Figure 58 Redness indicator a* as a function of days after slaughter by stimulation method (a – c) and muscles. Lines represent fits from a nonlinear mixed-effects model with varying parameters for electrical stimulation method based on an asymptotic regression function.

Table 19 Results from a multiple-comparison procedure following a nonlinear mixed-effects model with separate parameter estimates for each electrical stimulation method (values in brackets are standard errors of the parameter estimates). Model parameters: *Asym* = asymptote, maximum a* value, R_0 = response when the predictor is zero, *lrc* = logarithm of the rate for a* to attain maximum value. Different lower case letters indicate significant differences between parameter estimates at P < 0.05.

Stimulation method	Model parameters		
	Asym	R_0	*lrc*
Stimul 1	24.46 (0.29) [a]	17.11 (0.77) [a]	-1.06 (0.19) [a]
Stimul 2	25.82 (0.72) [a]	19.48 (0.47) [b]	-2.17 (0.30) [b]
Stimul 3	24.85 (0.29) [a]	17.27 (0.74) [a]	-1.11 (0.18) [a]

The pooled Stimul 1 and 3 analysis for a* as a function of days after slaughter showed significant differences between muscles (P < 0.001) (Figure 59). Apart from BLD, all muscles reached peak values in colour indicator 'a*' within 10 days of slaughter (Figure 59) and there was little variation in the maximum a* values (see parameter *Asym*, Table 20). Overall, all muscles had similar a* values to start with (parameter R_0 in Table 20). The rate to attain maximum 'a' values (parameter *lrc,* Table F) increased in a similar manner across muscles except for BLD muscle. This muscle demonstrated a more gradual increase and thus differed significantly in *lrc* from all muscles but RUMC (Table 20). The only significant difference occurred between KNUCK and RUMH, which showed the lowest and largest a* values, respectively.

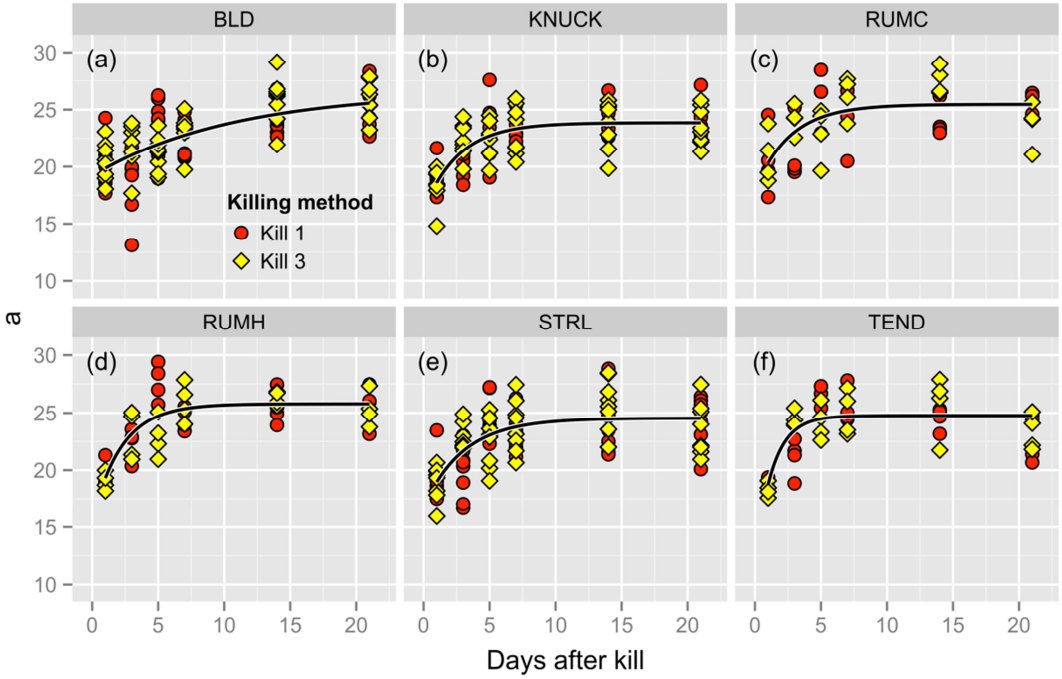

Figure 59 Redness indicator a* as a function of days after slaughter by muscles (a – f) and stimulation method (red circles = Stimul 1, yellow diamonds = Stimul 3). Lines represent fits from a nonlinear mixed-effects model with individual parameter estimates for each muscle based on an asymptotic regression function.

Table 20 Results from a pairwise-comparison procedure following an asymptotic nonlinear mixed-effects model for redness indicator a* with individual parameter estimates for each muscle (values in brackets are standard errors of the parameter estimates). Model parameters: *Asym* = asymptote, maximum a* value R_0 = a* when days after slaughter is zero, *lrc* = logarithm of the rate for a* to attain maximum value. Different lower case letters indicate significant differences between parameter estimates at P < 0.05.

Muscle	Model parameters (SE)		
	Asym	R_0	*lrc*
BLD	26.60 (1.32) [ab]	19.21 (0.62) [a]	-2.37 (0.43) [a]
KNUCK	23.85 (0.36) [a]	16.18 (1.72) [a]	-0.97 (0.26) [b]
RUMC	25.46 (0.51) [ab]	18.00 (1.74) [a]	-0.98 (0.39) [ab]
RUMH	25.79 (0.47) [b]	15.61 (1.91) [a]	-0.79 (0.31) [b]
STRL	24.54 (0.37) [ab]	16.79 (1.01) [a]	-1.11 (0.24) [b]
TEND	24.76 (0.40) [ab]	11.92 (3.92) [a]	-0.27 (0.39) [b]

For the stand-alone Stimul 2 slaughter, a model comparison between an asymptotic model and a quadratic model supported the latter, which was subsequently used ($AIC_{asymptotic}$ = 957 vs. $AIC_{quadratic}$ = 943). Muscles in the Stimul 2 analysis showed an expected more humped relationship between a* and days after slaughter, except for the *infraspinatus* (BLD) muscle. That muscle displayed an almost linear relationship (Figure 60).

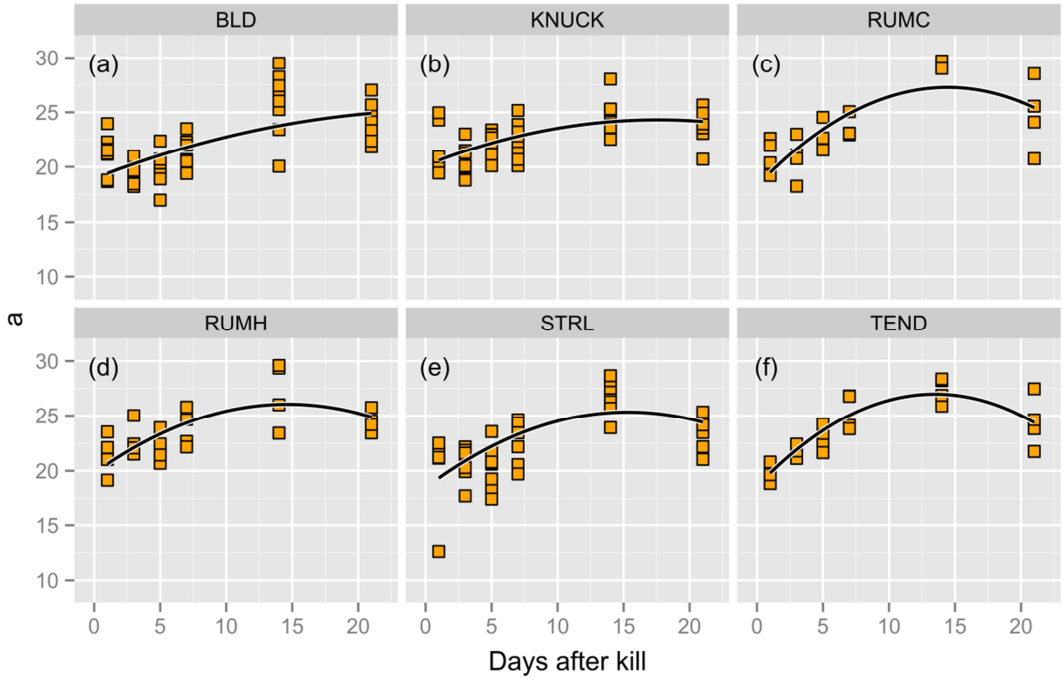

Figure 60 Redness indicator a* as a function of days after slaughter by muscles (a – f) for Stimul 2. Lines represent fits from a nonlinear mixed-effects model with individual parameter estimates for each muscle based on an alternative quadratic function.

There were no significant differences in the curvature among the muscles group (parameter α in Table 21). However, BLD and KNUCK muscles were excluded from pair-wise comparisons because their parameter estimates for the shape parameter α were not significant; whereas BLD was also excluded from pair-wise comparisons on β for the same reason. Overall, all muscles attained the highest a* value around 15 days of aging (refer to β values), except for BLD muscle. Its peak values were attained later. However, due to the large variation seen in the parameter estimate for BLD muscles, this difference was statistically not significant (see parameter β in Table 21 and Figure 60). The highest a* values were observed in RUMC ($\gamma = 27.27$)

and TEND ($\gamma = 26.97$) which did not differ from the maximum values in other muscles, except for KNUCK. This muscle showed the lowest maximal a* colour value of $\gamma = 24.30$.

Table 21 Results from a pairwise-comparison procedure following a nonlinear quadratic model for colour indicator a* with separate parameter estimates for each muscle within Stimul 2 (values in brackets are standard errors of the parameter estimates). Model parameters: α = shape parameter, β = days after slaughter (x-axis) when a* attained its maximum value, γ = maximum a* value. Different lower case letters indicate significant differences between parameter estimates at P < 0.05. Grey-shaded areas: BLD and KNUCK were excluded from pairwise comparisons for the shape parameter α (BLD was also excluded from β comparisons), because their estimate of α (β) was not significant.

Muscle	Model parameters (SE)		
	α	β	γ
BLD	-0.008 (0.008)	27.18 (14.84)	25.23 (1.78) [ab]
KNUCK	-0.013 (0.008)	17.61 (4.12) [a]	24.30 (0.51) [a]
RUMC	-0.043 (0.011) [a]	14.44 (1.07) [a]	27.27 (0.73) [b]
RUMH	-0.029 (0.011) [a]	14.66 (1.68) [a]	26.04 (0.73) [ab]
STRL	-0.029 (0.008) [a]	15.33 (1.30) [a]	25.32 (0.52) [ab]
TEND	-0.046 (0.011) [a]	13.49 (0.85) [a]	26.97 (0.77) [b]

The relationship between yellowness colour indicator b* and time after slaughter varied significantly across stimulation methods (P < 0.001, Figure 61). Specifically, there were significant differences in the curvature of the relationship (parameter α) and the maximum b* value between Stimul 2 and 3 (parameter γ, Table 22). Stimul 1 had an intermediate parameter estimates and thus did not differ significantly from the other stimulation methods. There were no significant differences between the stimulation methods in the time to reach maximum b* values, ranging from 14 to 17 days (parameter β, Table 22). Because there was a significant difference in the maximal b* values (parameter γ, Table 22), Stimul 1 and 2 were pooled for further analysis; whereas Stimul 3 was analysed in isolation.

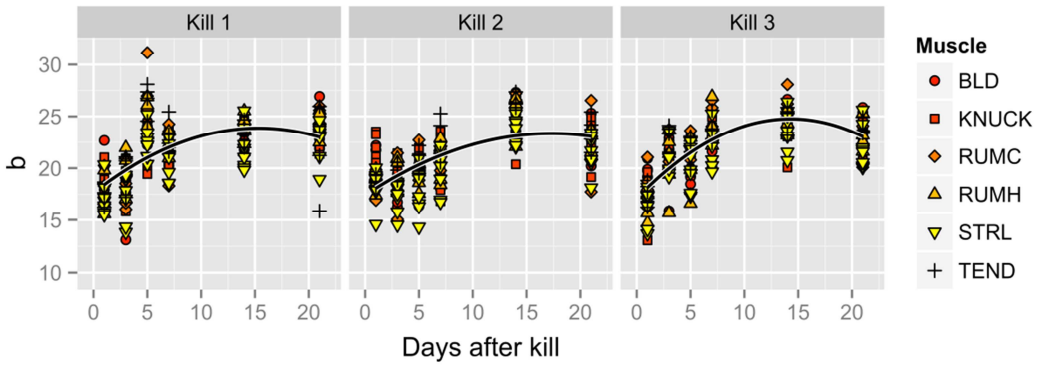

Figure 61 Yellowness indicator b* as a function of days after slaughter by stimulation method and muscle. The lines represent fits from a nonlinear mixed-effects model with varying parameters for stimulation method based on the alternative quadratic equation.

Table 22 Results from a pairwise-comparison procedure following a nonlinear model for colour indicator b* with separate parameter estimates for each stimulation method (values in brackets are standard errors of the parameter estimates). Model parameters: α = shape parameter, β = days after slaughter (x-axis) when b* value reached maximum, γ = maximum b* value. Different lower case letters indicate significant differences between parameter estimates at P < 0.05.

Stimulation method	Model parameters (SE)		
	α	β	γ
Stimul 1	-0.027 (0.004) [ab]	15.34 (0.75) [a]	23.86 (0.26) [a]
Stimul 2	-0.020 (0.004) [a]	17.32 (1.34) [a]	23.35 (0.25) [a]
Stimul 3	-0.037 (0.004) [b]	14.29 (0.44) [a]	24.75 (0.26) [b]

In the pooled analysis of Stimul 1 and 2 data for b*, the yellowness indicator, the curvature was similar for most muscles (Figure 62). The BLD and KNUCK showed the weakest curvature and differed significantly from TEND that displayed the strongest curvature (parameter α in Table 23, Figure 62). The time to reach maximum b* values ranged from 13 to nearly 20 days but did not differ significantly among muscles due to large variations (parameter β, Table 23). TEND showed the highest b* value, which was significantly larger than the values seen for BLD,

KNUCK and STRL but not significantly different from the values reached in RUMC and RUMH (parameter γ, Table 23, Figure 62).

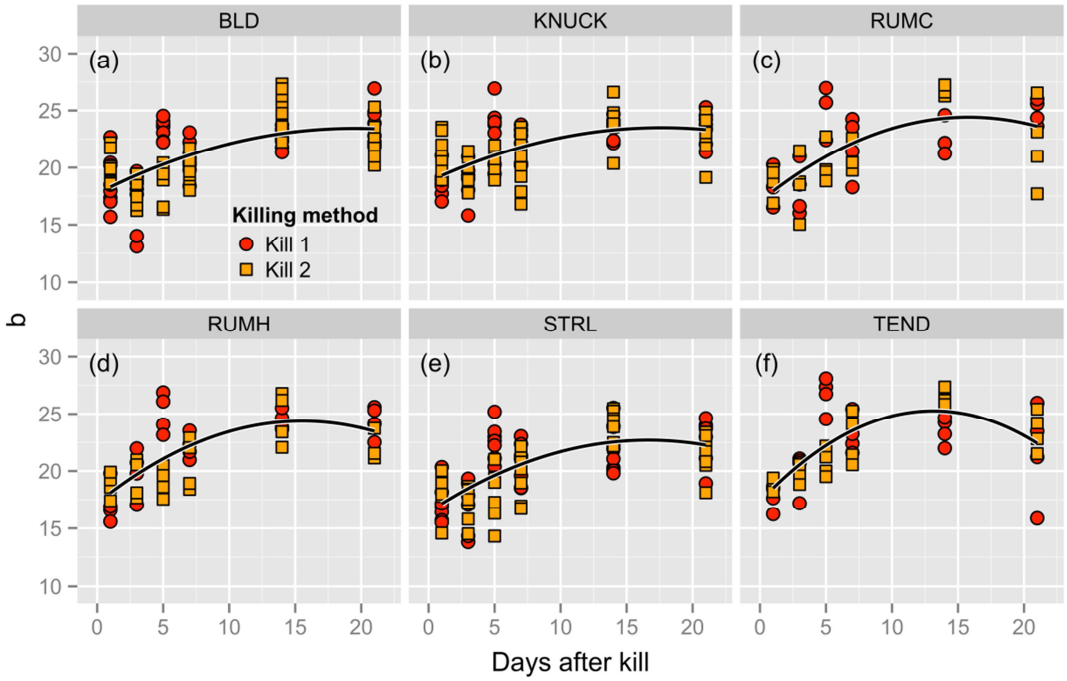

Figure 62 Colour indicator b* as a function of days after slaughter by muscles (a – f) and Stimul 1 and 2 (red circles = 1, orange squares = 2). Lines represent fits from a nonlinear mixed-effects model with individual parameter estimates for each muscle based on the alternative quadratic function.

Table 23 Results from a pairwise-comparison procedure following a nonlinear mixed-effects model yellowness indicator b* (pooled response for Stimul 1 and 2) with separate parameter estimates for each muscle (values in brackets are standard errors of the parameter estimates). Model parameters: α = shape parameter, β = days after slaughter (x-axis) when b* value reached a maximum, γ = maximum b* value. Different lower case letters indicate significant differences between parameter estimates at P < 0.05.

Muscle	Model parameters (SE)		
	α	β	γ
BLD	-0.015 (0.006) [a]	19.50 (3.49) [a]	23.43 (0.42) [ab]
KNUCK	-0.015 (0.006) [a]	17.65 (2.83) [a]	23.50 (0.38) [ab]
RUMC	-0.029 (0.008) [ab]	15.81 (1.54) [a]	24.42 (0.53) [ac]
RUMH	-0.030 (0.008) [ab]	15.52 (1.46) [a]	24.37 (0.53) [ac]
STRL	-0.023 (0.006) [ab]	16.64 (1.63) [a]	22.69 (0.37) [b]
TEND	-0.046 (0.008) [b]	13.14 (0.61) [a]	25.26 (0.57) [c]

In the isolated analysis of Stimul 3 data for b*, the yellowness indicator, the curvature was similar for most muscles (Figure 63).The model with individual parameters for each muscle provided a better fit than the restricted model with common parameter estimates suggesting significant differences across muscles (P < 0.01). However, the differences were not very large and after P-value correction for multiple testing, none of the pairwise comparisons was significant (Table 24). The maximum b* values of around 25 were reached about 14 days after slaughter (Figure 63, Table 24).

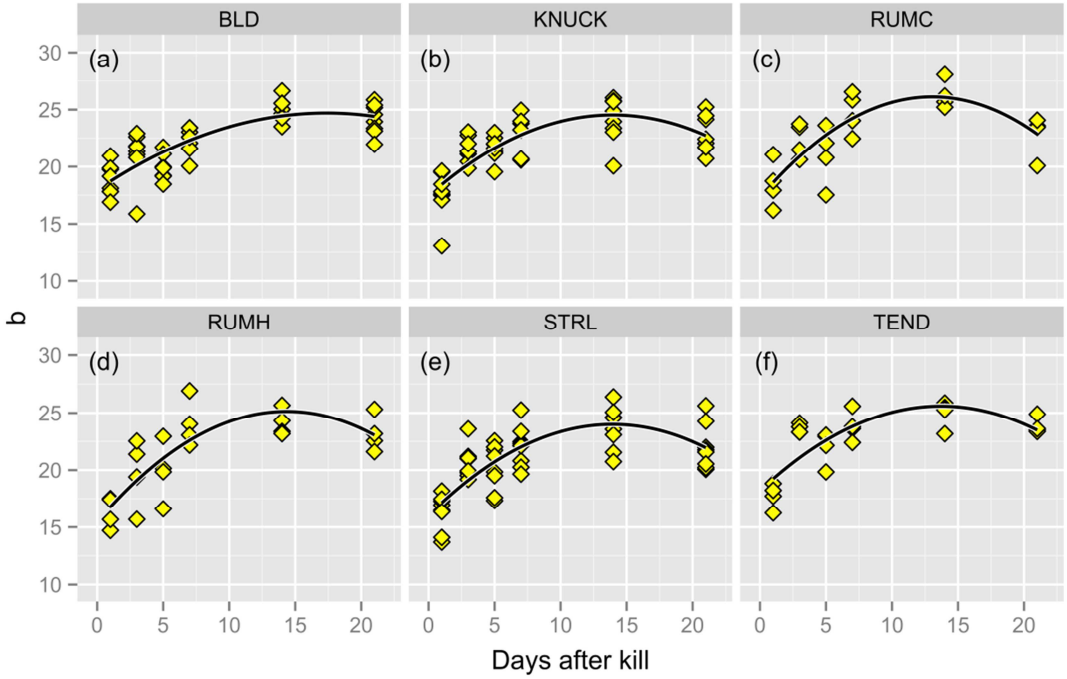

Figure 63 Colour indicator b* as a function of days after slaughter by muscles (a – f) for Stimul 3. Lines represent fits from a nonlinear mixed-effects model with individual parameter estimates for each muscle based on the alternative quadratic function.

Table 24 Results from a pairwise-comparison procedure following a nonlinear mixed-effects model of yellowness indicator b* for Stimul 3 with separate parameter estimates for each muscle (values in brackets are standard errors of the parameter estimates). Model parameters: α = shape parameter, β = days after slaughter (x-axis) when b* value reached a maximum, γ = maximum b*. Different lower case letters indicate significant differences between parameter estimates at P < 0.05.

Muscle	Model parameters (SE)		
	α	β	γ
BLD	-0.022 (0.007) [a]	17.37 (2.14) [a]	24.70 (0.44) [a]
KNUCK	-0.036 (0.007) [a]	13.95 (0.77) [a]	24.53 (0.47) [a]
RUMC	-0.052 (0.010) [a]	13.05 (0.63) [a]	26.12 (0.67) [a]
RUMH	-0.047 (0.010) [a]	14.35 (0.89) [a]	25.13 (0.66) [a]
STRL	-0.041 (0.007) [a]	13.94 (0.66) [a]	24.00 (0.47) [a]
TEND	-0.039 (0.010) [a]	13.70 (0.94) [a]	25.59 (0.67) [a]

Table 25 Rate of pH decline results from a multiple comparison procedure using Tukey contrasts

Linear hypotheses	Estimate	SE	*z*	*P*	Significant
RatepHtx					
2 – 1 = 0	-0.045	0.043	-1.025	0.557	
3 – 1 = 0	-0.136	0.054	-2.523	0.031	*
3 – 2 = 0	-0.092	0.045	-2.042	0.101	
RatepHtcon					
2 – 1 = 0	0.378	0.100	3.764	< 0.001	***
3 – 1 = 0	0.544	0.109	5.006	< 0.001	***
3 – 2 = 0	0.167	0.063	2.628	0.022	*

Table 26 Results from a generalised additive mixed model (GAMM) for hue angle applying individual time smoothers for each muscle. *edf* = estimated degrees of freedom. Different lower case letters indicate significant differences between smoothers (multiple-comparison procedure using Tukey contrasts). s = smoother term.

Parametric coefficients	Estimate (SE)	*t*	P
Intercept of Stimul 1	0.759 (0.003)	227.81	<0.001
Stimul 2	-0.010 (0.003)	-3.46	<0.001
Stimul 3	0.003 (0.003)	1.04	0.300
KNUCK	0.011 (0.003)	3.70	<0.001
RUMC	-0.016 (0.004)	-4.56	<0.001
RUMH	-0.026 (0.004)	-7.10	<0.001
STRL	-0.028 (0.003)	-8.71	<0.001
TEND	0.004 (0.003)	1.22	0.224
Approx. significance of smooth terms			
	edf	*F*	P
s(time) : BLD [a]	1.00	0.09	0.764
s(time) : KNUCK [b]	2.96	4.40	<0.01
s(time) : RUMC [c]	1.93	12.94	<0.001
s(time) : RUMH [c]	1.00	28.34	<0.001
s(time) : STRL [c]	3.20	36.97	<0.001
s(time) : TEND [b]	1.00	8.91	<0.001

Table 27 Results from a backwards selection testing the significance of the fixed terms of the linear mixed-effects model for L* in Stimul1 and 3 based on the AIC (Akaike Information Criterion) and likelihood ratio tests.

Eliminated term	AIC	Likelihood ratio	Degrees freedom	P
None (full model)	2069.89			
Stimul method × muscle × time	2066.71	6.82	5	0.234
Muscle × time	2065.68	8.97	5	0.110
Stimul method × time	2064.72	0.01	1	0.928
Stimul method × muscle	2061.99	5.28	5	0.383
Time	2059.95	3.10	1	0.078
Stimul method	2057.20	0.34	1	0.558
Muscle	2226.82	177.97	5	<0.001

Table 28 Results from a backwards selection testing the significance of the fixed terms of the linear mixed-effects model for Japan in Stimul 1 and 3 based on the AIC (Akaike Information Criterion) and likelihood ratio tests.

Eliminated fixed term	AIC	Likelihood ratio	Degrees freedom	P
None (full model)	761.92			
Stimul method × muscle × time	758.58	6.66	5	0.247
Muscle × time	756.32	7.74	5	0.171
Stimul method × time	756.61	0.03	1	0.860
Stimul method × muscle	753.86	5.28	5	0.382
Time	747.89	0.29	1	0.589
Stimul method	748.06	0.47	1	0.494
Muscle	920.36	180.77	5	<0.001

Table 29 Results from a GAMM for meat shear force applying individual time smoothers for each muscle. edf = estimated degrees of freedom. All t and F distribution values were larg e and highly significant. Different lower case letters indicate significant differences between parameter estimates or between smoother, by a multiple-comparison procedure using Tukey contrasts.

Muscle	Estimated mean (SE)	t	P
BLD	5.36 (0.10) [a]	54.84	<0.001
KNUCK	5.96 (0.10) [b]	59.33	<0.001
RUMC	5.15 (0.12) [a]	43.43	<0.001
RUMH	6.48 (0.12) [c]	55.22	<0.001
STRL	5.98 (0.10) [b]	58.64	<0.001
TEND	4.98 (0.12) [a]	42.91	<0.001
Approximate significance of smoother terms			
	edf	F	P
s(time): BLD [a]	2.33	18.39	<0.001
s(time): KNUCK [b]	2.96	14.80	<0.001
s(time): RUMC [a]	1.93	21.96	<0.001
s(time): RUMH [c]	1.00	16.96	<0.001
s(time): STRL [d]	3.20	46.92	<0.001
s(time): TEND [e]	1.00	5.03	0.025